



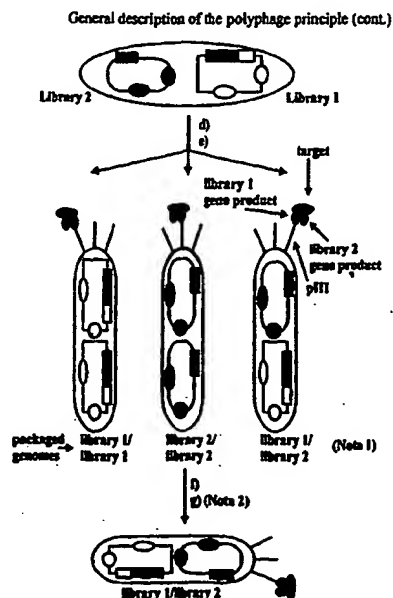
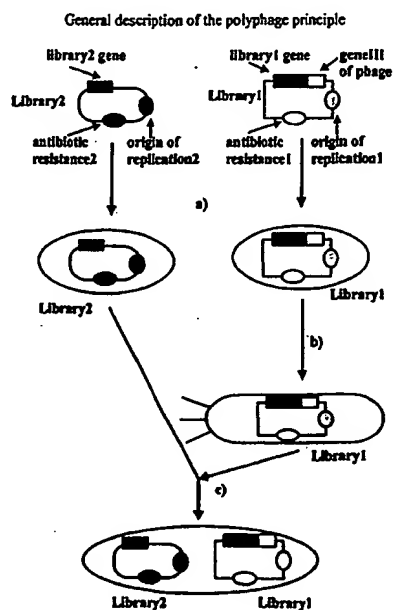
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|----|---|
| (51) International Patent Classification ⁶ : C12Q | A2 | (11) International Publication Number: WO 99/06587 (43) International Publication Date: 11 February 1999 (11.02.99) |
| (21) International Application Number: PCT/EP98/04836 (22) International Filing Date: 3 August 1998 (03.08.98) (30) Priority Data: 97113319.4 1 August 1997 (01.08.97) EP (71) Applicant (for all designated States except US): MORPHOSYS GESELLSCHAFT FÜR PROTEINOPTIMIERUNG AG [DE/DE]; Am Klopferspitz 19, D-82152 Martinsried (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): RUDERT, Fritz [DE/DE]; Josef-Retzner-Strasse 36, D-81241 München (DE). GE, Liming [CN/DE]; Portiastrasse 12, D-81545 München (DE). ILAG, Vic [PH/DE]; Knorrstrasse 85, D-89897 München (DE). (74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, D-81675 München (DE). | | (81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published Without international search report and to be republished upon receipt of that report. |

(54) Title: NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX



(57) Abstract

The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|---------------------|----|-----------------------|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Larvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | Republic of Macedonia | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's | NZ | New Zealand | | |
| CM | Cameroon | | Republic of Korea | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakistan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

NOVEL METHOD AND PHAGE FOR THE IDENTIFICATION OF NUCLEIC ACID SEQUENCES ENCODING MEMBERS OF A MULTIMERIC (POLY)PEPTIDE COMPLEX

The present invention relates to methods for the identification of nucleic acid sequences encoding members of a multimeric (poly)peptide complex by screening for polyphage particles. Furthermore, the invention relates to products and uses thereof for the identification of nucleic acid sequences in accordance with the present invention.

Since its first conception by Ladner in 1988 (WO88/06630), the principle of displaying repertoires of proteins on the surface of phage has experienced a dramatic progress and has resulted in substantial achievements. Initially proposed as display of single-chain Fv (scFv) fragments, the method has been expanded to the display of bovine pancreatic trypsin inhibitor (BPTI) (WO90/02809), human growth hormone (WO92/09690), and of various other proteins including the display of multimeric proteins such as Fab fragments (WO91/17271; WO92/01047).

A Fab fragment consists of a light chain comprising a variable and a constant domain (VL-CL) non-covalently binding to a heavy chain comprising a variable and constant domain (VH-CH1). In Fab display one of the chains is fused to a phage coat protein, and thereby displayed on the phage surface, and the second is expressed in free form, and on contact of both chains, the Fab assembles on the phage surface.

Various formats have been developed to construct and screen Fab phage-display libraries. In its simplest form, just one repertoire, e. g. of heavy chains, is encoded on the phage or phagemid vector. A corresponding light chain, or a repertoire of light chains, is expressed separately. The Fab fragments assemble either inside a host cell, if the light chain is co-expressed from a plasmid, or outside the cell in the medium, if a collection of secreted phage particles each displaying a heavy chain is contacted with the light chain(s) expressed from a different host cell. By screening such Fab libraries, just the information about the heavy chain encoded on the phage or phagemid vector is retrievable, since that vector is packaged in the phage particle. By reverting the format and displaying a library of light chains, and

assembling Fab fragments by co-expressing or adding one or more of the heavy chains identified in the first round, corresponding light chain-heavy chain pairs can be identified.

To avoid that multi-step procedure, both repertoires may be cloned into one phage or phagemid vector, one chain expressible as a fusion with at least part of a phage coat protein, the second expressible in free form. After selection, the phage particle will contain the sequence information about both chains of the selected Fab fragments. The disadvantage of such a format is that the overall complexity of the library is limited by transformation efficiency. Therefore, the library size will usually not exceed 10^{10} members.

For various applications, a library size of up to 10^{14} would be advantageous. Therefore, methods of using site-specific recombination, either based on the Cre/lox system (WO92/20791) or on the att λ system (WO 95/21914) have been proposed. Therein, two collection of vectors are sequentially introduced into host cells. By providing the appropriate recombination sites on the individual vectors, recombination between the vectors can be achieved by action of an appropriate recombinase or integrase, achieving a combinatorial library, the overall library size being the product of the sizes of the two individual collections. The disadvantages of the Cre/lox system are that the recombination event is not very efficient, it leads to different products and is reversible. The att λ system leads to a defined product, however, it creates one very large plasmid which has a negative impact on the production of phages. Furthermore, the action of recombinase or integrase most likely leads to undesired recombination events.

Thus, the technical problem underlying the present invention is to develop a simple, reliable system which enables the simultaneous identification of members of a multimeric (poly)peptide complex, such as the identification of heavy and light chain of a Fab fragment, in phage display systems.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims. Accordingly, the present invention allows to easily create and screen large libraries of multimeric (poly)peptide complexes for properties such as binding to a target, as in the case of screening Fab fragment libraries, or such as enzymatic activity, as in the case of libraries of multimeric enzymes. The technical approach of the present invention, i.e. the retrieval of information about two members of a multimeric (poly)peptide complex

encoded on two different vectors without requiring a recombination event, is neither provided nor suggested by the prior art.

Accordingly, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, said method being characterized by screening or selecting for polyphage particles that contain said combination.

Surprisingly, it has been achieved by the present invention that the phenomenon of polyphages can be used to co-package the genetic information of two or more members of multimeric (poly)peptide complexes in a phage display system. The occurrence of polyphage particles has been observed 30 years ago (Salivar et al., Virology 32 (1967) 41-51), where it was described that approximately 5% of a phage population form particles which are longer than unit length and which contain two or more copies of phage genomic DNA. They occur naturally when a newly forming phage coat encapsulates two or more single-stranded DNA molecules. In specific cases, it has been seen that co-packaging of phage and phagemids or single-stranded plasmid vectors takes place as well (Russel and Model, J. Virol. 63 (1989) 3284-3295). Despite of occasional scientific articles about the morphogenesis of polyphage particles, a practical application has never been discussed or even been mentioned. In WO92/20791 in example 26, a model experiment for a combinatorial Fab display library expressed from separate vectors is presented. However, there is only a screening process for either of the two vectors described. Thus, the prior art teaches away from screening for the simultaneous presence of two vectors in a polyphage particle.

In the context of the present invention, the term "multimeric (poly)peptide complex" refers to a situation where two or more (poly)peptide(s) or protein(s), the "members" of said multimeric complex, can interact to form a complex. The interaction between the individual members will usually be non-covalent, but may be covalent, when post-translational modification such as the formation of disulphide-bonds between any two members occurs. Examples for "multimeric (poly)peptide complexes" comprise structures such as fragments derived from immunoglobulins (e. g. Fv, disulphide-linked Fv (dsFv), Fab fragments), fragments derived from other members of the immunoglobulin superfamily (e.g. α , β -

heterodimer of the T-cell receptor), and fragments derived from homo-or heterodimeric receptors or enzymes. In phage display, one of said members is fused to at least part of a phage coat protein, whereby that member is displayed on, and assembly of the multimeric complex takes place at, the phage surface. A "combinatorial phage library" is produced by randomizing at least two members of said multimeric (poly)peptide complex at least partially on the genetic level to create two libraries of genetically diverse nucleic acid sequences in appropriate vectors, by combining the libraries in appropriate host cells and by achieving co-expression of said at least two libraries in a way that a library of phage particles is produced wherein each particle displays one of the possible combinations out of the two libraries.

By screening such a combinatorial phage library displaying multimeric (poly)peptide complexes for a predetermined property, a collection of phage particles will be identified. Partially, these particles will just contain the genetic information of one of the members of the multimeric complex. The inventive principle of the present invention is the screening step for polyphage particles containing the genetic information of a combination of library members.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

- (a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
- (b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry

or encode a second selectable and/or screenable property different from said first property;

- (c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;
- (d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
- (e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
- (f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
- (g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);
- (h) identifying said combination of nucleic acid sequences.

Optionally, further members of said multimeric complex may be provided in the case of ternary, quaternary or higher (poly)peptide complexes. These further members may, for example, be co-expressed from one of the phage or phagemid vectors or from a separate vector such as a plasmid. Even libraries of such further members could be employed in which case further screenable or selectable properties would have to be introduced on the corresponding vectors. Alternatively, such further libraries could be contained in said first or second libraries of recombinant vector molecules. In another option, further screening and/or selection steps or a repetition of the individual steps can be carried out, to optimize the result of obtaining and identifying said nucleic acid sequences.

Furthermore, the present invention relates to a method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes, comprising the steps of

- (a) expressing in appropriate host cells under appropriate conditions

- (aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
 - (ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
 - (ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,
- so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
- (b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
 - (c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
 - (d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);
 - (e) identifying said combination of nucleic acid sequences.

In a preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.

In a further preferred embodiment of the method of the present invention, the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.

In a most preferred embodiment of the method of the present invention said two phagemid vectors are compatible.

The term "compatibility" refers to a property of two phagemids to be able to coexist in a host cell. Incompatibility is connected to the presence of incompatible plasmid origins of replication belonging to the same incompatibility group. An example for compatible plasmid origins of replication is the high-copy number origin ColE1 and the low-copy number origin p15A.

Therefore, in a further preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.

In a most preferred embodiment of the method of the present invention, said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.

It could be shown, that two phagemids both having a ColE1-derived plasmid origin of replication can coexist in a cell as long as one of the ColE1 origins carries a mutation.

Particularly preferred is a method, wherein said vectors and/or said helper phage comprise different phage origins of replication.

Most preferred is an embodiment of the method of the present invention, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.

The term "interference" refers to a property that phagemids inhibit the production of progeny phage particles by interfering with the replication of the DNA of the phage. "Interference resistance" is a property which overcomes this problem. It has been found that mutations in the intergenic region and/or in gene II contribute to interference resistance (Enea and Zinder, Virology 122 (1982), 222-226; Russel et al., Gene 45 (1986) 333-338). It was identified that phages called IR1 and IR2 (Enea and Zinder, Virology 122 (1982), 222-226), and mutants derived therefrom such as R176 (Russel and Model, J. Bacteriol. 154 (1983) 1064-1076), R382, R407 and R408 (Russel et al., Gene 45 (1986) 333-338) and R383 (Russel and Model, J. Virol. 63 (1989) 3284-3295) are interference resistant by carrying mutations in the untranslated region upstream of gene II and in the gene II coding region.

Therefore, in a preferred embodiment of the method of the present invention, said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.

In a most preferred embodiment said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

In a further embodiment of the method of the invention, said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.

In the context of the present invention, the term "hybrid nucleic sequences" refers to vector elements which comprise sequences originating from different phage(mid) vectors.

Surprisingly, it has been found that a vector constructed combining a part derived from fd phage and a second part derived from R408, a derivative of f1 phages, is interference resistant and additionally, gives predominantly polyphage particles.

Therefore, a most preferred embodiment of the method of the present invention relates to a vector which is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.

In a yet further preferred embodiment of the method according to the present invention, said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in an additional preferred embodiment to a method, wherein said derivative is a phagemid comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

The invention relates in a further preferred embodiment to a method, wherein said derivative is a helper phage comprising essentially the phage origin or replication from fpep3_1B-

IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Most preferred is an embodiment of the method of the invention, wherein said derivatives comprise the combined fd/fl origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

The formation of polyphage particles has been examined in more detail by different groups. It was found that amber mutations in genes VII and IX lead to the amplified production of infectious polyphage particles (Lopez and Webster, Virology 127 (1983) 177-193). A couple of mutants in gene VII (R68, R100) and in gene IX (N18) were identified and further characterized.

Accordingly, in a preferred embodiment of the method of the present invention, the gene VII contained in any of said vectors contains an amber mutation, and most preferably, said mutation is identical to those found in phage vectors R68 or R100.

Further preferred is an embodiment, wherein the gene IX contained in any of said vectors contains an amber mutation, and most preferably said mutation is identical to that found in phage vector N18.

Several phage coat proteins have been used in displaying foreign proteins including the gene III protein (gIIIp), gVIp, and gVIIIp.

In a preferred embodiment of the method of the present invention, said phage coat protein is gIIIp or gVIIIp.

In a particularly preferred embodiment of the method of the present invention, said phage particles are infectious by having a full-length copy of gIIIp.

The gIIIp is a protein comprising three domains. The C-terminal domain is responsible for membrane insertion, the two N-terminal domains are responsible for binding to the F pilus of *E. coli* (N2) and for the infection process (N1).

In a most preferred embodiment of the method of the invention, said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the

displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.

In the context of the present invention, the term "infectivity-mediating particle" (IMP) refers to a construct comprising either the N1 domain or the N1-N2 domain. On interaction with a non-infectious phage lacking said domains, infectivity of the phage particles can be restored. The interaction between the non-infectious phage and the IMP can be mediated by a ligand fused to the IMP, which can bind to a partner displayed on the phage. By screening a non-infectious phage display library against a target ligand-IMP construct, restoration of infectivity can be used to select target-binding library members.

In a further preferred embodiment of the method of the invention, said truncated gIIIp comprises the C-terminal domain of gIIIp.

In a yet preferred embodiment of the method of the invention, said truncated gIIIp is derived from phage fCA55.

In addition to the work by Lopey and Webster cited above, Crissman and Smith (Virology 132 (1984) 445-455) could show, that the phage fCA55 which has a large deletion in gene III removing the N-terminal domains and a large part of the C-terminal domain leads exclusively to the formation of polyphages.

Particularly preferred is an embodiment of the method of the invention, wherein said predetermined property is binding to a target.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of an immunoglobulin.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the present invention relates to a method, wherein said predetermined property is the activity to perform or to catalyze a reaction.

In a preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is an enzyme.

In a most preferred embodiment of the method of the invention, said multimeric (poly)peptide complex is a fragment of a catalytic antibody.

In a further most preferred embodiment of the method of the invention, said fragment is an Fv, dsFv or Fab fragment.

An additional preferred embodiment of the invention relates to a method, wherein selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.

In a most preferred embodiment of the method of the invention, said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.

Particularly preferred is a method, wherein said identification of said nucleic acid sequences is effected by sequencing.

Further preferred is a method, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives, TG1, XL1kann or TOP10F.

An additional preferred embodiment of the invention relates to a polyphage particle which

(a) contains

(i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex

fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and

(ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that carries or encodes a second selectable and/or screenable property different from said first property;

and (b) displays said multimeric (poly)peptide complex at its surface.

A most preferred embodiment of the invention relates to a polyphage particle, wherein said phage coat protein is the gIIIp.

A further preferred embodiment of the present invention relates to a polyphage particle which is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.

Additionally, the invention relates to a polyphage particle which is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.

Most preferably, the invention relates to the phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.

Additionally preferred, the invention relates to a phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Further preferred is an embodiment of the invention, which relates to a phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Preferably, the invention relates to a helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.

Additionally preferred is an embodiment, said derivatives comprise the combined fd/fl origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

Further preferred is the use of any of the vectors according to the present invention in the generation of polyphage particles containing a combination of at least two different vectors.

Most preferred is the use of vectors of the invention, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.

Further preferred in the present invention is the use of vectors, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

Legends to Figures:

Figure 1: General description of the polyphage principle for the display of a Fab library: e.g. library 1: library of VL chains; library 2: VH chains; both libraries on compatible phagemids; in a: libraries are transformed into host cells; in b: library 1 is rescued by a helper phage; in c: libraries are combined by infection; in d: co-expression of heavy and light chains; in e: rescue by helper phages, production of phage particles, assembly of Fab on phage, selection for target; note 1: A certain fraction of the phage particles will be normal unit-length particles containing just one of the two genomes (not shown in Figure 1). Furthermore, polyphage does not discriminate which genomes to package. Therefore, the combinations shown in Figure 1 can arise. To select for

correctly packaged genomes, the subsequent steps are required; in f: infect host cells; in g: select for ability to confer resistance to two antibiotics to infected cells; note 2: only phage that satisfy condition according to g) represent polyphage particles which contain the correct combination of heavy and light chain of binding Fabs (Hetero-polyphage). Unit-length phage as well as polyphage carrying two identical genomes will confer only resistance to one antibiotics.

- Figure 2: Functional map and sequence of phage vector fhag1A
- Figure 3: Functional map and sequence of phage vector fjun_1B
- Figure 4: Functional map and sequence of phage vector fppep3_1B-IR3seq
- Figure 5: Compatibility of various phage and phagemid vectors: co-transformation of different vector pairs and growth in liquid culture (can/amp selection):
 A. fjun_1B-R408-IR/pIG10_pep10; B. fjun_1B/pIG10_pep10 (only 1 colonie);
 C. fppep3_1B-IR3/pIG10_pep10; D. fjun_1B-R408-IR/pOK1Djun; E. fjun_1B/pOK1Djun: no growth; F. fppep3_1B-IR3/pOK1Djun;
 a. fjun_1B; b. fjun_1B-R408-IR; c. fppep3_1B-IR3; d. pIG10_pep10; e. pOK1Djun
- Figure 6: co-transformation of positive (pep3/p75ICD combination, lane 9) and negative (jun/p75ICD, lane 10) pairs; lane 1 to 8: SIP transductants
- Figure 7: Sensitivity of SIP hetero-polyphage system for selection in solution: #SIP hetero-polyphage transductants, transducing units (t.u.)/ml, produced by co-cultures of co-transformants as in Figure 6 mixed at the indicated ratios.
- Figure 8: PCR to identify phage vector(s) present in SIP polyphage transductants: lane 1 to 6: SIP polyphage transductants; lane A: fppep3_1B-IR3/pIG10.3-IMPp75 co-transformant; lane B: fjun_1B-IR3/pIG10.3-IMPp75 co-transformant
- Figure 9: IR Phage and Phagemid are Co-packaged into Polyphages: 1: Δ gIII phage + gIII plasmid; 2: IR phage+ phagemid
- Figure 10: SIP Information is Co-transduced by Polyphages: a: IMPp75 on phage vector; b: pep10-gIII-CT fusion on phage vector; c: IMPp75 on phagemid vector; d: pep10-gIII-CT fusion on phagemid vector

The examples illustrate the invention

Example 1: Selection for polyphage transductants

In WO92/01047, page 83, a model experiment for a two-vector system is described which uses a phage vector (fd-CAT2-IV) encoding a light chain and a phagemid vector (pHEN1-III) encoding a heavy chain. The phagemid, grown in *E. coli* HB2151, was rescued with fd-CAT2-IV phage, and functional phage(mid)s produced. By infecting TG1 cells and plating on tetracycline (to select for fd-CAT) and ampicillin (to select for pHEN1), the ratio of phage and phagemid being packaged was determined.

By repeating this experiment, but plating on TYE plates with both antibiotics, polyphage transductants transducing both resistances simultaneously can be selected, and the genetic information contained on the phage and phagemid vector can be retrieved.

By replacing the single light and heavy chain in the constructs mentioned above by corresponding repertoires, a library of Fab-displaying phage particles can be produced. By screening that library against an immobilized target, a collection of phage particles can be identified. Polyphage particles contained in that collection can be identified by transducing both resistances as described above.

Example 2: Generation and use of an interference-resistant filamentous phage to co-package the genetic information of co-displayed interacting proteins

Introduction

The physical connection of randomly combined genetic information is of vital importance in processes such as interactive screening of two libraries of expressed protein members or for co-expression and co-display of protein pairs which are dependent on the interaction with each other for proper function.

2.1.: Construction of a interference resistant filamentous phage:

2.1.1.: Construction of fjun_1B:

- fha1A (see Figure 2)

- a. The phage vector f17/9-hag (Krebber *et al.*, 1995, *FEBS Letters* 377, 227-231) is digested with EcoRV and XmnI. The 1.1 kb fragment containing the anti-HAG Ab gene is isolated

by agarose gel electrophoresis and purified with a Qiagen gel extraction kit. This fragment is ligated into a pre-digested pIG10.3 vector (EcoRV-XmnI). Ligated DNA is transformed into DH5a cells and positive clones are verified by restriction analysis. The recombinant clone is called **pIGhag1A**. All cloning described above and subsequently are according to standard protocols (Sambrook *et al.*, 1989, *Molecular Cloning: a Laboratory Manual*, 2nd ed.)

- b. The vector f17/9-hag (Krebber *et al.*, 1995) is digested with EcoRV and StuI. The 7.9 kb fragment is isolated and self-ligated to form the vector **fhag2**.
- c. The chloramphenicol resistance gene (CAT) assembled *via* assembly PCR (Ge and Rudolph, *BioTechniques* 22 (1997) 28-29) using the template pACYC (Cardoso and Schwarz, *J. Appl. Bacteriol.* 72 (1992) 289-293) is amplified by the polymerase chain reaction (PCR) with the primers:
CAT_BspEI(for): 5' GAATGCTCATCCGGAGTTC
CAT_Bsu36I(rev): 5' TTTCAGTGGCCTCAGGCTAGCACCAGGCGTTTAAG
- d. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with BspEI and Bsu36I then ligated into pre-digested fhag2 vector (BspEI-Bsu36I; 7.2 kb fragment) to form **fhag2C**.
- e. The vector fhag2C is digested with EcoRI and the ends made blunt by filling-in with Klenow fragment. The flushed vector is self-ligated to form vector **fhag2CdelEcoRI**.
- f. pIGhag1A is digested with XbaI and HindIII. The 1.3 kb fragment containing the anti-HAG gene fused with the C-terminal domain of filamentous phage pIII protein is isolated and ligated with a pre-digested fhag2CdelEcoRI phage vector (XbaI-HindIII; 6.4 kb) to create the vector **fhag1A**.

- fjun_1B (see Figure 3)

- a. The DNA encoding the C-terminal domain including the long linker separating it from the amino terminal domain of the filamentous phage pIII (gIII short) is amplified by PCR using pOK1 (Gramatikoff *et al.*, *Nucleic Acids Res.* 22 (1994) 5761-5762) as template with the primers:
gIII short(for): 5'GCTTCCGGAGAATTCAATGCTGGCGGCGGCTCT3'
gIII short(rev): 5'CCCCCACAAGCTTATCAAGACTCCTTATTACG3'
- b. The PCR is done following standard protocols (Sambrook *et al.*, 1989). The amplified product is digested with EcoRI and HindIII, then ligated into pre-digested fhag1A vector (EcoRI-HindIII) to form the vector **fjun_1B**.

2.1.2.: Construction of fjun_1B-R408IR:

In order to introduce mutations which have been described to confer an interference resistance phenotype (Enea and Zinder, Virology 122 (1982), 222-226) into the non-interference resistant fd phage vector fjun_1B (see Fig.3), a 1.7 kb fragment of helper phage R408 (Stratagene) comprising the region between the unique restriction sites *DraIII* and *BsrGI* was PCR amplified by assembly PCR. Subfragments of the 1.7 kb *DraIII*/*BsrGI* fragment were amplified from the f1 phage R408 template DNA with primer combinations FR604/FR605 and FR606/FR607 to introduce via the partially complementary primers FR605 and FR606 an additional *gII* mutation found to be present in the recipient construct fjun_1B. Resulting PCR fragments were gel-purified and combined to serve as template in an subsequent assembly PCR with primers FR604 and FR607. PCR conditions were standard, with approx. 25 ng template, 10 pmole of each primer, 250 pmole of each dNTP, 2 mM Mg, 2.5 U Pfu DNA polymerase (Stratagene). Amplification was done for 30 cycles, with 1 min denaturation at 94 C, 1 min annealing at 50°C, 1 min extension at 72°C. The correct-sized 1.7 kb assembly PCR product was gel-purified, digested with *DraIII* and *BsrGI* and cloned into *DraIII*/*BsrGI*-digested fjun_1B, generating fjun_1B-R408IR.

Primers: FR604 5' GTTCACGTAGTGGGCCATCG 3'
FR605 5' TGAGAGGTCTAAAAAGGCTATCAGG 3'
FR606 5' TAGCCTTTTGTAGACCTCTCAAAAATAG 3'
FR607 5' CGGTGTACAGACCAGGCGC 3'

2.2.: Proof of principle experiments

Despite of the absence of the two originally associated IR mutations, the hybrid phage vector fjun_1B-R408IR (carrying the chloramphenicol acetyltransferase conferring chloramphenicol resistance) could be co-transformed with a phagemid (pOK1deltajun, carrying the beta-lactamase gene conferring ampicillin resistance) containing a phage origin of replication. More importantly, fjun_1B-R408IR could stably co-exist with the phagemid pOK1deltajun, and the phagemid was efficiently co-packaged together with the fjun_1B-R408IR phage genome into polyphage particles. Titers of polyphages, simultaneously

transducing chloramphenicol and ampicillin resistance, reached 6×10^8 transducing units (t.u.)/ml of overnight bacterial culture K91 plating cells, a number almost equivalent to a titer of 10^9 /ml seen after selection on chloramphenicol only. Selection of the K91 transductants on ampicillin only gave a titer of 5×10^9 /ml. These titers indicated that more than 50 % of all phages containing fjun_1B-R408IR also contained the phagemid pOK1deltajun, thus representing polyphages. This high ratio of polyphages was confirmed by restriction analysis of transductants which had been selected on chloramphenicol only. More than 50 % of these clones also contained the phagemid in addition to the fjun_1B-R408IR phage genome. fjun_1B-R408IR was isolated in pure form from an individual transductant, which contained only this phage. The construct fjun_1B-R408IR was used with pOK1deltajun for co-transformation of DH5 α cells, in order to produce selectively-infective phages (SIP) via fos-jun leucine zipper interaction (which non-covalently restores wt gIII function). Stable, double-resistant co-transformants were obtained with this combination and individual clones were grown overnight in the presence of cam/amp. The culture supernatant of these clones was filtered through a 45 μ M membrane filter and used to infect exponentially-growing F+ bacteria (K91 strain) for 20 min at 37 C. To test for the presence of infective SIP polyphages the cells were plated on LB agar plates containing cam and amp and plates were incubated at 37 C overnight. Approx. 500 to 1000 transforming units (t.u.)/ml resulting in double-resistant transductants were obtained from individual co-transformants. DNA of those transductants was analyzed by restriction analysis which showed that 95 % (15/16 clones) of the clones had the correct pattern expected for fjun_1B-R408IR and pOK1deltajun. Supernatants of several polyphage transductants were tested for persistent SIP phage production by re-infection of K91 cells. This confirmed that polyphage transductants continued to produce infective SIP phages and restriction analysis of the resulting 2nd round polyphage transductants showed that 44 % (14/32 clones) contained the correct vector combination. The rest of the clones contained the correct pOK1deltajun phagemid plus a recombined phage vector with a restored wt gIII, indicating an increase in recombination frequency when both vectors are propagated in the rec+ strain K91 (compared to the rec- strain DH5 α used for co-transformation of IR phage and phagemid). To test other protein-protein interactions which give a higher titer of infective SIP phages and to verify the presence of hetero-polyphages (co-packaging of phage and phagemid instead of co-infection by monophages or homo-polyphages), two peptide ligands (previously selected by SIP, WO97/32017)

which bind to the p75 rat neurotrophin receptor (Chao et al., Science 232 (1986) 518-521) intracellular domain (p75ICD) were cloned as N-terminal gIIIc fusions in fjun_1B-R408IR (replacing jun) and the phagemid pIG10.3, leading to constructs fpep3_1B-IR3seq and pIG10.3-pep10 (WO97/32017), respectively, which contain the peptide pep3: 5'-TGTATTGTTTATCATGCTCATTATCTTGTTGCTAAGTGT-3' encoding the amino acid sequence (CysIleValTyrHisAlaHisTyrLeuValAlaLysCys) instead of the jun sequence. Sequencing of the respective parts of the transferred R408 fragment in fpep3_1B-IR3seq revealed that neither of the two IR mutations (the G5986>A mutation from complementation group I in the gII 5' non-translated region, which should be found at position 3225 in fpep3_1B-IR3seq, and the C143>T mutation (3789 in fpep3_1B-IR3seq) from complementation group II leading to a Thr>Ile amino acid exchange in gII) were found to be present. However, the gII mutation G6090>T (3329 in fpep3_1B-IR3seq), leading to a Leu>Val exchange, introduced by assembly PCR was present. Furthermore, three additional mutations compared to an f1 phage could be identified: G5737>A (2976 in fpep3_1B-IR3seq) in the phage origin of replication, G343>A (3989) in gII, and G601>T (4247) in gII/X.

The functional map and the sequence of fpep3_1B-IR3seq are given in Figure 4. This sequence was double-checked several times. It could be shown that differences in the sequence of fpep3_1B-IR3seq compared to published sequence data could be explained by mutations already present in the starting constructs used for cloning fjun_1B-R408IR and fpep3_1B-IR3seq.

Co-transformation experiments (Fig. 5) using combinations of pIG10.3 or pOK1 phagemids (both with f1 oris) with fjun_1B ("wt" fd phage), fjun_1B-R408-IR (containing the DraIII/BsrGI fragment from R408) or fpep3_1B-IR3 (containing the DraIII/BsrGI fragment from R408 and the PCR mutation) revealed that the PCR mutation is not necessary for the IR phenotype, at least judged by the ability to be co-transformable with a phagemid and the ability of individual co-transformants to grow in liquid culture (cam/amp selection).

Additionally, the interacting protein partner p75ICD was cloned as a C-terminal fusion to the infectivity-mediating domains (N1-N2) of gIII (infectivity-mediating particle (IMP) fusion) resulting in constructs fIMPp75-IR3 and pIG10.3-IMPp75.

The IR phage was tested with the SIP pairing fpep3_1B-IR3seq3/ pIG10.3-IMPp75 (which gives a higher titer than fos/jun SIP) in the presence of the negative control combination fjun_1B-IR3seq3/ pIG10.3-IMPp75 (Fig. 6). A SIP hetero-polyphage titer of 1.5×10^5 /ml (cam/amp-resistant transductants) was achieved with fpep3_1B-IR3seq3/ pIG10.3-IMPp75. To test SIP sensitivity in a model library vs. library setting, co-transformants of fpep3_1B-IR3seq3/ pIG10.3-IMPp75 were diluted in an excess fjun_1B-IR3/ pIG10.3-IMPp75 and the supernatant of the bacterial co-culture was assayed for SIP hetero-polyphages. This showed that down to a dilution of 10^{-5} to 10^{-6} can be recovered (Fig. 7).

To prove that only the correct phage vector is present in SIP polyphage transductants, DNA of positive (fpep3_1B-IR3seq3/ pIG10.3-IMPp75) and negative (fjun_1B-IR3/ pIG10.3-IMPp75) control co-transformants, as well as DNA from the SIP polyphage transductants derived from SIP phages produced by the mix of positive and negative control bacteria was analyzed by PCR (Fig. 8). Primers FR614 (5'-GCTCTAGATAACGAGGGC-3') and FR627 (5'-CGCAAGCTTAAGACTCCT-TATTACGC-3') amplify the phage region from the start of ompA to the end of gIII. PCR products derived from fpep3_1B-IR3seq3 and fjun_1B-IR3 can be discriminated by size. Gel analysis of the above samples verified that only the expected fpep3_1B-IR3seq3 phage was present in SIP polyphage transductants (6 analyzed).

To physically demonstrate the existence of hetero-polyphages (which have phage and phagemid co-packaged) when using the IR phage vector, phages produced by co-transformants of fIR3/pIG10.3-IMPp75 and as a control fjun_1B/JB61 ("wt" phage plus complementing gIII plasmid) were separated on an agarose gel (Fig. 9). This showed that the fIR3/pIG10.3-IMPp75 combination produced substantially more slower migrating (thus bigger) phages than the fjun_1B/JB61 control combination. The ratio was almost inversed. Elution of phages from various regions of the gel and subsequent titering of the eluate on plating cells showed that the upper gel region contained a significant portion of double resistance-transducing phages which thus can be regarded as hetero-polyphages.

The pairs fpep3_1B-IR3 and pIG10.3-IMPp75 as well as fIMPp75-IR3 and pIG10.3-pep10 were co-transformed into DH5 α , individual cam/amp resistant clones were grown and the culture supernatant was tested on K91 cells for SIP phage production (Fig. 10). The combinations fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10 gave a titer of 1.5×10^5 t.u./ml and 5×10^3 t.u./ml, respectively when assayed for cam/amp-resistant transductants. The titer for each combination when assayed on LB cam was nearly the same as when assayed on LB cam/amp. This demonstrated efficient co-packaging of phage and phagemid DNA to almost 100 %, as seen before with the initial fjun_1B-R408IR and pOK1deltajun combination. To proof the existence of polyphages which individually co-transduce phage and phagemid DNA simultaneously, and to rule out the possibility of transduction of the two resistance markers by independent (and thus random) co-infection by two different phages which have only phage or phagemid packaged, a statistical test was performed. Defined, identical aliquots of bacterial culture supernatants of an individual co-transformant representing each of the two SIP vector combinations described above (fpep3_1B-IR3/pIG10.3-IMPp75 and fIMPp75-IR3/pIG10.3-pep10) were either used individually to infect K91 cells followed by selection on LB cam and LB amp plates, or the same supernatant aliquots from the two vector combinations were mixed before infection of K91 cells and selection on LB cam/amp. 117 cam-resistant, 328 amp-resistant and 141 cam/amp-resistant transforming units were present in the supernatant aliquot from the fIMPp75-IR3/pIG10.3-pep10 combination and 40 cam-resistant, 30 amp-resistant and 23 cam/amp-resistant transforming units were present in the supernatant aliquot from the fpep3_1B-IR3/pIG10.3-IMPp75 combination. The mix of both supernatant aliquots contained 166 cam-resistant and 162 cam/amp-resistant transforming units, exactly corresponding to the expected numbers which would be obtained by adding up the transducing units of the two individual aliquots. 48 cam/amp-resistant transductant colonies were picked from the plate where the mix of the two individual aliquots was used for infection and were analyzed by restriction digest. This showed that only the correct, SIP phage-producing vector combination (5 clones containing the fpep3_1B-IR3/pIG10.3-IMPp75 and 43 clones containing the fIMPp75-IR3/pIG10.3-pep10 combination; this represents a ratio of the two input vector combinations in the analyzed transductants of 1 : 8.6 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10), which is very similar to the 1 : 6.1 (fpep3_1B-IR3/pIG10.3-IMPp75 : fIMPp75-IR3/pIG10.3-pep10) ratio of double-resistant input phages in this experiment) occurred in all analyzed

transductants, verifying the presence of hetero-polyphages by ruling out the possibility of random co-infection and thus incorrect, random combination by two out of four possible monophage and/or homo-polyphage populations (fpep3_1B-IR3, pIG10.3-IMPp75, fIMPp75-IR3 and pIG10.3-pep10) each containing only one type of vector (phage or phagemid). Statistically, co-infection of the same bacterium by two separate phages was practically already excluded by the small numbers of infective phages containing at least one resistance marker (166 cam-resistant and 358 amp-resistant phages) which were used in the above experiment. Co-infection of the same bacterium (of a total of 10^7 bacteria) by one of the 166 cam-resistant phages and one of the 358 amp-resistant phages has a probability of 6×10^{-10} . Moreover, in this scenario incorrect combinations of individual phage and phagemid vectors (e.g. fpep3_1B-IR3/ pIG10.3-pep10 and fIMPp75-IR3/ pIG10.3-IMPp75) would be possible. The fact that only the correct vector combinations were found in all 48 transductants analyzed from this experiment further proved that co-transduction by hetero-polyphage and not random co-infection by homo-polyphage or monophage was the mechanism by which double-resistance was transduced.

2.3.: Construction of a phage-display system for Fab display

The constructs described in 3.2. can easily be modified to achieve the display of Fabs or a Fab library. In fpep3_1B-IR3seq, the jun part can be replaced by a VL-CL light chain repertoire having the appropriate 3'- and 5'-restriction sites similarly as described for pep_3-to construct fVL_1B-R408IR. In pIG10.3-IMPp75, the IMPp75 construct can be replaced by a repertoire of VH-CH1 heavy chains. After co-transformation of both repertoires into host cells and expression, a library of phage particles displaying Fab fragments is produced. Since fpep3_1B-IR3seq was set up for a SIP experiment by having just the C-terminal domain of gIII, the corresponding Fab-displaying phage particles are non-infectious. By adding a target molecule fused to an infectivity-mediating particle (N1-N2 domain of gIIp), phages displaying target-binding Fab fragments can be selected by infecting host cells.

By replacing the truncated gIII part described above by a full-length copy of gIII, a Fab-display library of infectious phage particles is obtained, which can be screened against immobilized targets. Binding phages can be eluted and used to infect host cells.

By selecting for transductants conferring cam/amp-resistance to their host cells, polyphage infections can be selected in both cases. Thereby the information about both chains of the selected Fab fragments can be retrieved.

CLAIMS

1. A method for identifying a combination of nucleic acid sequences encoding two members of a multimeric (poly)peptide complex with a predetermined property, said combination being contained in a combinatorial library of phage particles displaying a multitude of multimeric (poly)peptides complexes,
said method being characterized by screening or selecting for polyphage particles that contain said combination.
2. The method of claim 1, comprising the steps of
 - (a) providing a first library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to, and displayed at, the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
 - (b) providing a second library of recombinant vector molecules containing genetically diverse nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules of said second library are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
 - (c) optionally, providing nucleic acid sequences encoding further members of a multimeric (poly)peptide complex;
 - (d) expressing members of said libraries of recombinant vectors mentioned in steps (a), (b), and optionally nucleic acid sequences mentioned in step (c), in appropriate host cells under appropriate conditions, so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
 - (e) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
 - (f) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said

- multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;
- (g) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (f);
- (h) identifying said combination of nucleic acid sequences.

3. The method of claim 1, comprising the steps of

(a) expressing in appropriate host cells under appropriate conditions

- (aa) genetically diverse nucleic acid sequences contained in a first library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, said fusion protein thereby being able to be directed to and displayed at the phage surface, wherein said vector molecules are able to be packaged in a phage particle and carry or encode a first selectable and/or screenable property;
- (ab) genetically diverse nucleic acid sequences contained in a second library of recombinant vector molecules, said nucleic acid sequences comprising a variety of nucleic acid sequences, each encoding a second member of a multimeric (poly)peptide complex, wherein the vector molecules are able to be packaged in a phage particle and carry or encode a second selectable and/or screenable property different from said first property;
- (ac) optionally, nucleic acid sequences encoding further members of a multimeric (poly)peptide complex,
- so that a combinatorial library of phage particles each displaying a multimeric (poly)peptide complex is produced;
- (b) identifying in said library of phage particles a collection of phages displaying multimeric (poly)peptide complexes having said predetermined property;
- (c) identifying in said collection polyphage particles simultaneously containing recombinant vector molecules encoding a first and a second member of said multimeric (poly)peptide complex by screening or selecting for the simultaneous presence or generation of said first and second selectable and/or screenable property;

- (d) optionally, carrying out further screening and/or selection steps or repeating steps (a) to (c);
- (e) identifying said combination of nucleic acid sequences.
4. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of a phage vector and a phagemid vector.
 5. The method of anyone of claims 1 to 3, wherein the vectors of said first and said second library are a combination of two phagemid vectors, said appropriate conditions comprising complementation of phage genes by a helper phage.
 6. The method of claim 5, wherein said two phagemid vectors are compatible.
 7. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a p15A plasmid origin of replication.
 8. The method of claim 6, wherein said two phagemid vectors comprise a ColE1 and a mutated ColE1 origin.
 9. The method of anyone of claims 4 to 8, wherein said vectors and/or said helper phage comprise different phage origins of replication.
 10. The method of anyone of claim 4 to 9, wherein said phage vector, said phagemid vector(s) and/or said helper phage are interference resistant.
 11. The method of claim 10, wherein said phage vector, said phagemid vector(s) and/or said helper phage have mutations in the phage intergenic region(s), preferably in positions corresponding to position 5986 of f1, and/or in gene II, preferably in positions corresponding to position 143 of f1.
 12. The method of anyone of claims 10 to 11, wherein said phage vector, said phagemid vector(s) and/or said helper phage are, or are derived from, IR1 mutants such as R176, R382, R383, R407, R408, or from IR2 mutants.

13. The method of anyone of claims 4 to 11, wherein said vectors and/or said helper phage comprise hybrid nucleic acid sequences of f1, fd, and/or M13 derived sequences.
14. The method of anyone of claims 1 to 13, wherein said vector is, or is derived from, fpep3_1B-IR3seq with the sequence listed in Figure 4.
15. The method of claim 14, wherein said derivative is a phage comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
16. The method of claim 14, wherein said derivative is a phagemid comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
17. The method of claim 14, wherein said derivative is a helper phage comprising essentially the phage origin of replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
18. The method of anyone of claims 15 to 17, said derivatives comprise the combined fd/f1 origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.
19. The method of anyone of claims 1 to 18, wherein the gene VII contained in any of said vectors contains an amber mutation.
20. The method of claim 19, wherein said mutation is identical to those found in phage vectors R68 or R100.
21. The method of anyone of claims 1 to 20, wherein the gene IX contained in any of said vectors contains an amber mutation.

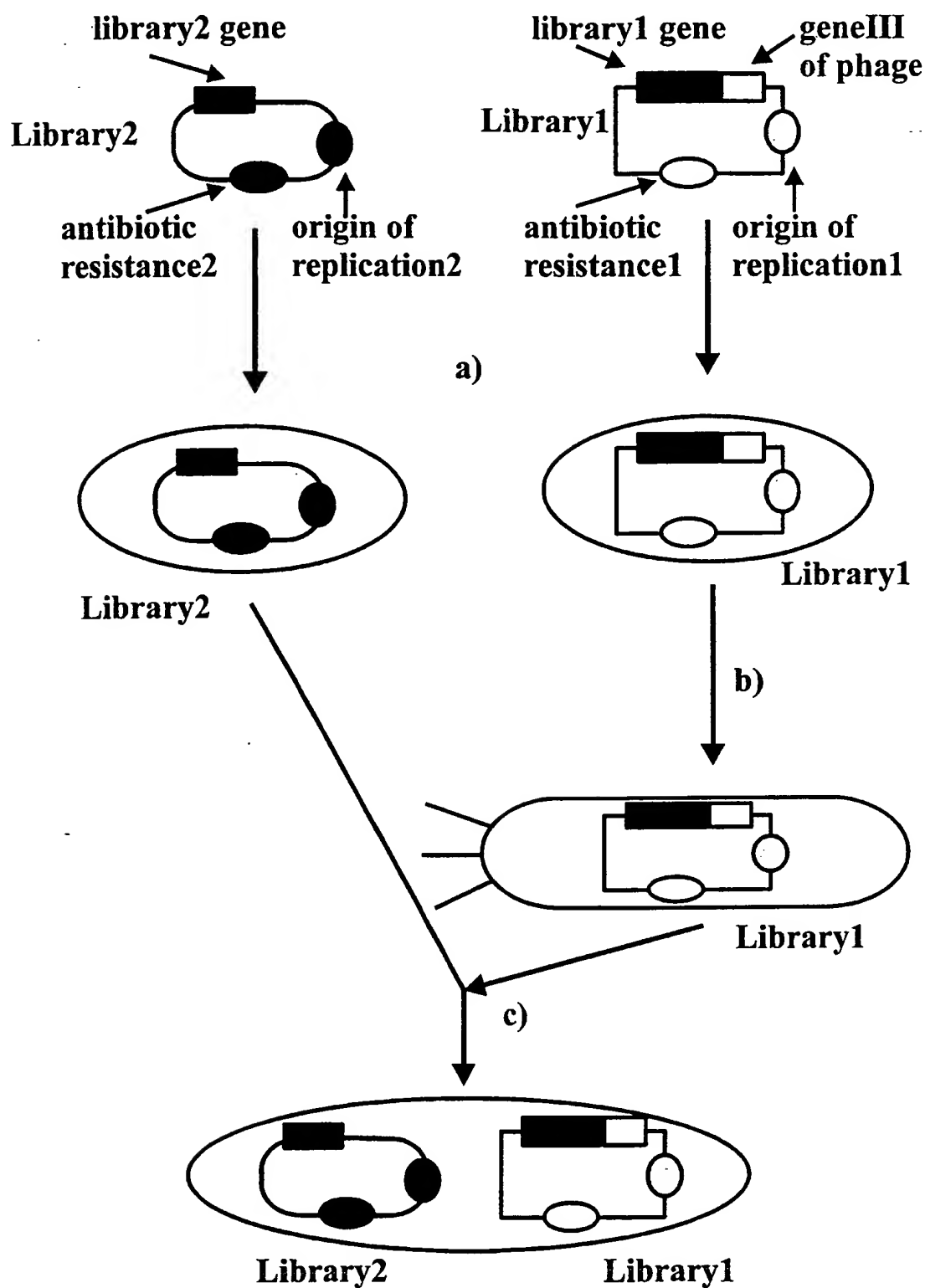
22. The method of claim 21, wherein said mutation is identical to that found in phage vector N18.
23. The method of anyone of claims 1 to 22, wherein said phage coat protein is gIIIp or gVIIIp.
24. The method of anyone of claims 1 to 23, wherein said phage particles are infectious by having a full-length copy of gIIIp.
25. The method of anyone of claims 1 to 24, wherein said phage particles are non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complexes with a corresponding partner coupled to an infectivity-mediating particle.
26. The method of claim 25, wherein said truncated gIIIp comprises the C-terminal domain of gIIIp.
27. The method of claim 26, wherein said truncated gIIIp is derived from phage fCA55.
28. The method of anyone of claims 1 to 27, wherein said predetermined property is binding to a target.
29. The method of claim 28, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin superfamily member.
30. The method of claim 29, wherein said multimeric (poly)peptide complex is a fragment of an immunoglobulin.
31. The method of claim 30, wherein said fragment is an Fv, dsFv or Fab fragment.
32. The method of anyone of claims 1 to 27, wherein said predetermined property is the activity to perform or to catalyze a reaction.

33. The method of claim 32, wherein said multimeric (poly)peptide complex is an enzyme.
34. The method of claim 33, wherein said multimeric (poly)peptide complex is a fragment of a catalytic antibody.
35. The method of claim 34, wherein said fragment is an Fv, dsFv or Fab fragment.
36. The method of anyone of claims 1 to 35, wherein said selectable and/or screenable property is the transactivation of transcription of a reporter gene such as beta-galactosidase, alkaline phosphatase or nutritional markers such as his3 and leu, or resistance genes giving resistance to an antibiotic such as ampicillin, chloramphenicol, kanamycin, zeocin, neomycin, tetracycline or streptomycin.
37. The method of anyone of claims 1 to 36, wherein said generation of said first and second screenable and/or selectable property is achieved after infection of appropriate host cells by said collection of phage particles.
38. The method of anyone of claims 1 to 37, wherein said identification of said nucleic acid sequences is effected by sequencing.
39. The method of anyone of claims 1 to 38, wherein said host cells are E.coli XL-1 Blue, K91 or derivatives thereof, TG1, XL1kann or TOP10F.
40. A polyphage particle which
- (a) contains
 - (i) a first recombinant vector molecule that comprises a nucleic acid sequence, which encodes a fusion protein of a first member of a multimeric (poly)peptide complex fused to at least part of a phage coat protein, and that carries or encodes a first selectable and/or screenable property, and
 - (ii) a second recombinant vector molecule that comprises a nucleic acid sequence, which encodes a second member of a multimeric (poly)peptide complex, and that

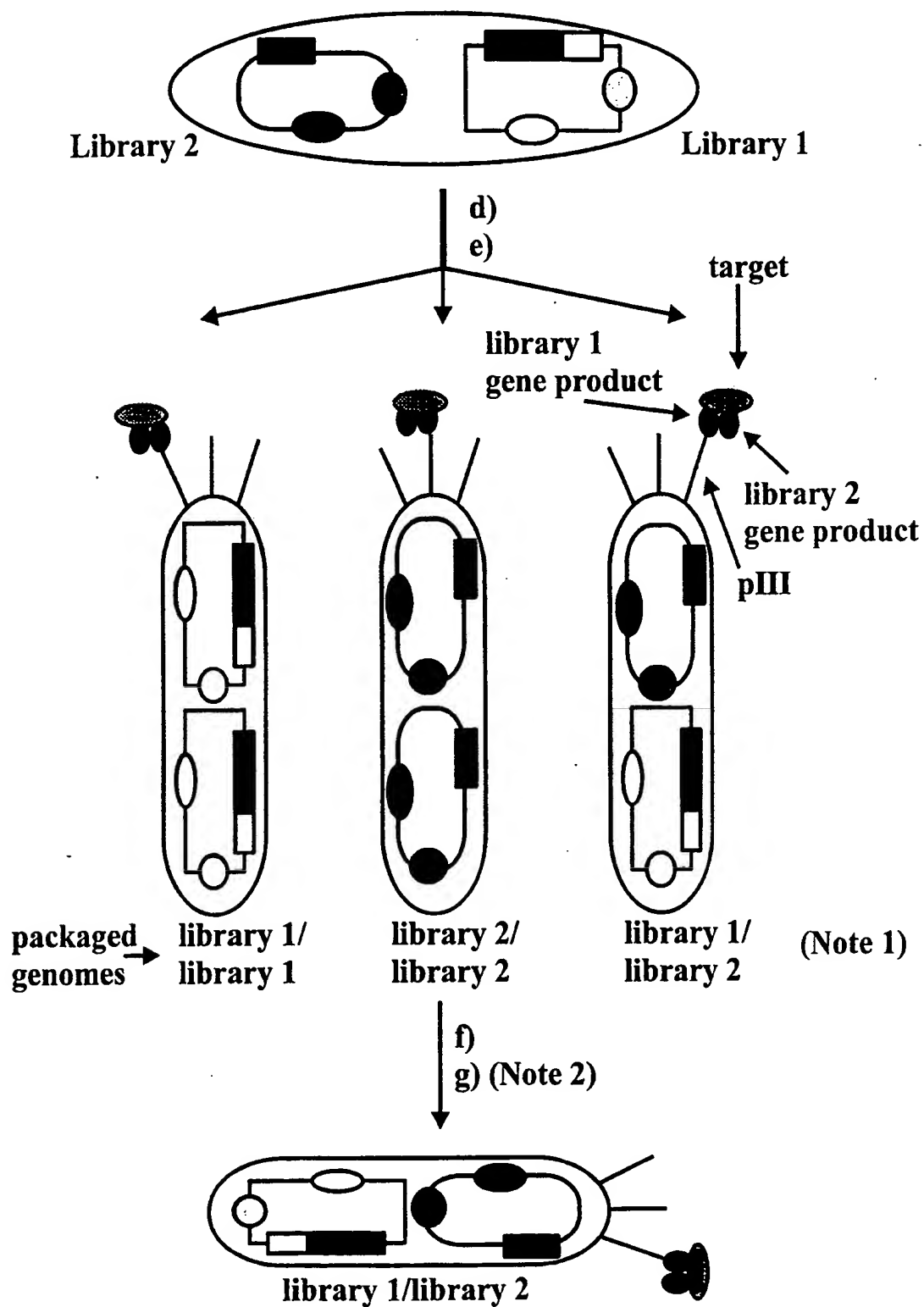
- carries or encodes a second selectable and/or screenable property different from said first property;
- and (b) displays said multimeric (poly)peptide complex at its surface.
41. The polyphage particle according to claim 40 wherein said phage coat protein is the gIIIp.
42. The polyphage particle according to claim 41 wherein said particles is infectious by having a full-length copy of gIIIp present, either in said fusion protein, or in an additional wild-type copy.
43. The polyphage particle according to claim 41 wherein said particles is non-infectious by having no full-length copy of gIIIp, said fusion protein being formed with a truncated version of gIIIp, wherein the infectivity can be restored by interaction of the displayed multimeric (poly)peptide complex with a corresponding partner coupled to an infectivity-mediating particle.
44. The phage vector fpep3_1B-IR3seq with the sequence listed in Figure 4.
45. A phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
46. A phagemid vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
47. A helper phage vector derived from phage vector fpep3_1B-IR3seq comprising essentially the phage origin or replication from fpep3_1B-IR3seq, the gene II from fpep3_1B-IR3seq, or a combination of said phage origin of replication and said gene II.
48. A vector according to anyone of claims 45 to 47, wherein said derivatives comprise the combined fd/fl origin including the mutation G5737>A (2976 in fpep3_1B-IR3seq), and/or the mutations G343>A (3989) in gII, and G601>T (4247) in gII/X.

49. The use according to any of the vectors of anyone of claims 44 to 48 in the generation of polyphage particles containing a combination of at least two different vectors.
50. The use according to claim 49, wherein said combination of different vectors comprises nucleic acid sequences encoding members of a multimeric (poly)peptide complex.
51. The use according to claim 50, wherein said combination of different vectors comprises nucleic acid sequences encoding interacting (poly)peptides/proteins.

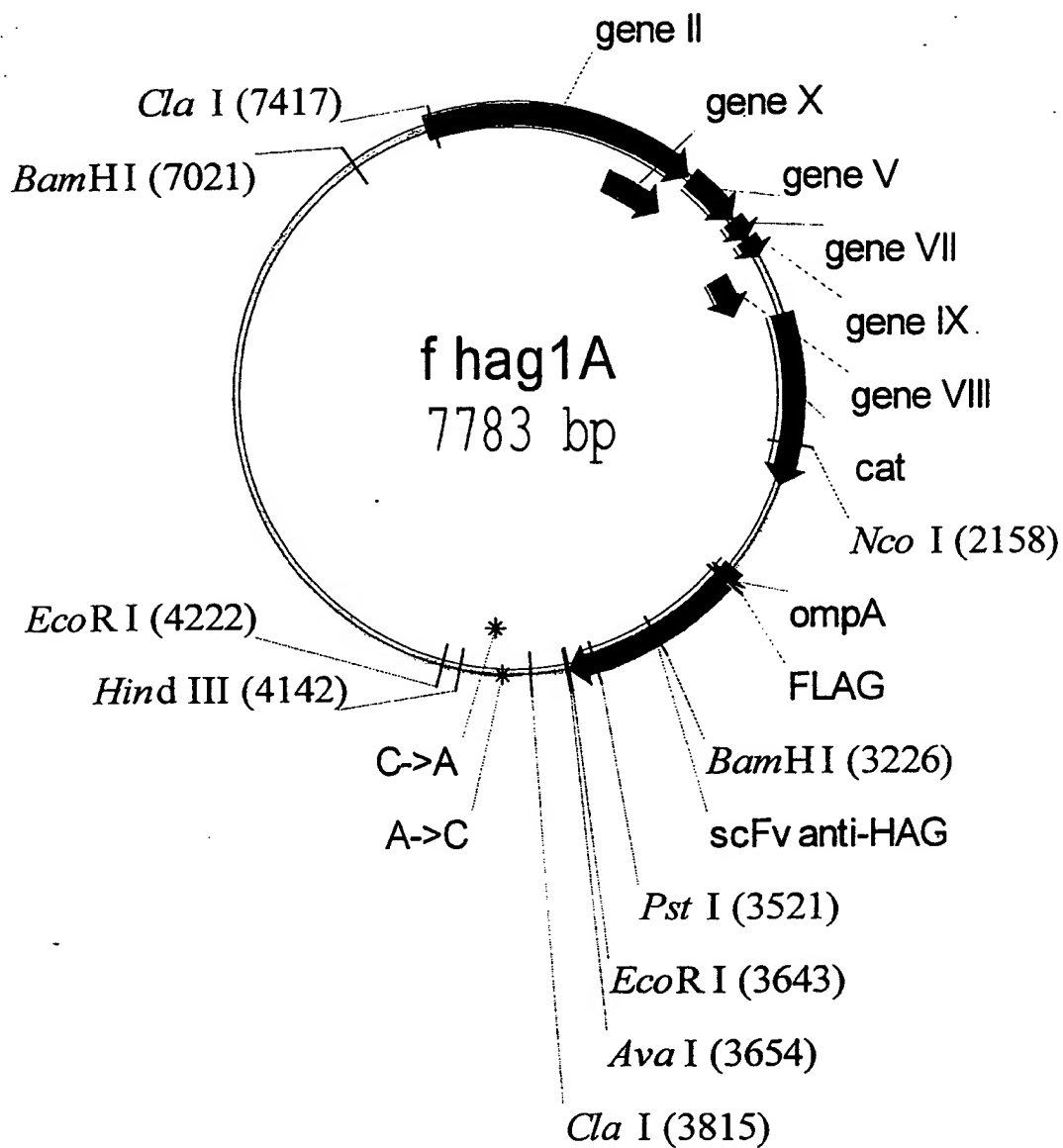
1/39

Figure 1: General description of the polyphage principle

2/39

Figure 1: General description of the polyphage principle (cont.)

3/39

Figure 2

4/39

| | | | | | |
|-----|------------|------------|------------|-------------|------------|
| 1 | AACGCTACTA | CCATTAGTAG | AATTGATGCC | ACCTTTTCAG | CTCGCGCCCC |
| | TTGCGATGAT | GGTAATCATC | TTAACTACGG | TGGAAGAGTC | GAGCGCGGGG |
| 51 | AAATGAAAAT | ATAGCTAAAC | AGGTTATTGA | CCATTTGCGA | AATGTATCTA |
| | TTTACTTTTA | TATCGATTGG | TCCAATAACT | GGTAAACGCT | TTACATAGAT |
| 101 | ATGGTCAAAC | TAAATCTACT | CGTTCGCAGA | ATTGGGAATC | AACTGTTACA |
| | TACCAGTTTG | ATTTAGATGA | GCAAGCGTCT | TAACCCTTAG | TTGACAATGT |
| 151 | TGGAATGAAA | CTTCCAGACA | CCGTACTTTA | GTTGCATATT | TAAAACATGT |
| | ACCTTACTTT | GAAGGTCTGT | GGCATGAAAT | CAACGTATAA | ATTTTGTACA |
| 201 | TGAACTACAG | CACCAGATTC | AGCAATTAAG | CTCTAAGCCA | TCCGCAAAAA |
| | ACTTGATGTC | GTGGTCTAAG | TCGTTAATTC | GAGATTCGGT | AGGCGTTTTT |
| 251 | TGACCTCTTA | TCAAAAGGAG | CAATTAAAGG | TACTGTCTAA | TCCTGACCTG |
| | ACTGGAGAAT | AGTTTTCTCT | GTTAATTTCC | ATGACAGATT | AGGACTGGAC |
| 301 | TTGGAATTTG | CTTCCGGTCT | GGTTCGCTTT | GAGGCTCGAA | TTGAAACGCG |
| | AACCTTAAAC | GAAGGCCAGA | CCAAGCGAAA | CTCCGAGCTT | AACCTTTCGC |
| 351 | ATATTTGAAG | TCTTTCGGGC | TTCTCTTTAA | TCTTTTTGAT | GCAATTCGCT |
| | TATAAACTTC | AGAAAGCCCG | AAGGAGAATT | AGAAAACTA | CGTTAAGCGA |
| 401 | TTGCTTCTGA | CTATAATAGA | CAGGGTAAAG | ACCTGATTTT | TGATTTATGG |
| | AACGAAGACT | GATATTATCT | GTCCCATTTT | TGGACTAAAA | ACTAAATACC |
| 451 | TCATTCTCGT | TTTCTGAACT | GTTTAAAGCA | TTTGAGGGGG | ATTCAATGAA |
| | AGTAAGAGCA | AAAGACTTGA | CAAATTTCTG | AAACTCCCCC | TAAGTTACTT |
| 501 | TATTTATGAC | GATTCCGCAG | TATTGGACGC | TATCCAGTCT | AAACATTTTA |
| | ATAAATACTG | CTAAGGCGTC | ATAACCTGCG | ATAGGTCAGA | TTTGTAAAT |
| 551 | CAATTACCCC | CTCTGGCAAA | ACTTCCTTTG | CAAAAGCCTC | TCGCTATTTT |
| | GTTAATGGGG | GAGACCGTTT | TGAAGGAAAC | GTTTTTCGGAG | AGCGATAAAA |
| 601 | GGTTTCTATC | GTCGTCTGGT | TAATGAGGGT | TATGATAGTG | TTGCTCTTAC |
| | CCAAAGATAG | CAGCAGACCA | ATTACTCCCA | ATACTATCAC | AACGAGAATG |
| 651 | CATGCCTCGT | AATTCCTTTT | GGCGTTATGT | ATCTGCATTA | GTTGAGTGTG |
| | GTACGGAGCA | TTAAGGAAAA | CCGCAATACA | TAGACGTAAT | CAACTCACAC |
| 701 | GTATTCCTAA | ATCTCAATTG | ATGAATCTTT | CCACCTGTAA | TAATGTTGTT |
| | CATAAGGATT | TAGAGTTAAC | TACTTAGAAA | GGTGGACATT | ATTACAACAA |
| 751 | CCGTTAGTTC | GTTTTATTAA | CGTAGATTTT | TCCTCCCAAC | GTCCTGACTG |
| | GGCAATCAAG | CAAAATAATT | GCATCTAAAA | AGGAGGGTTG | CAGGACTGAC |
| 801 | GTATAATGAG | CCAGTTCTTA | AAATCGCATA | AGGTAATTCA | AAATGATTAA |
| | CATATTACTC | GGTCAAGAAT | TTTAGCGTAT | TCCATTAAGT | TTTACTAATT |

5/39

| | | | | | |
|------|-------------|-------------|-------------|-------------|------------|
| 851 | AGTTGAAATT | AAACCGTCTC | AAGCGCAATT | TACTACCCGT | TCTGGTGTTT |
| | TCAACTTTAA | TTTGGCAGAG | TTCGCGTTAA | ATGATGGGCA | AGACCACAAA |
| 901 | CTCGTCAGGG | CAAGCCTTAT | TCACTGAATG | AGCAGCTTTG | TTACGTTGAT |
| | GAGCAGTCCC | GTTTCGGAATA | AGTGACTTAC | TCGTCGAAAC | AATGCAACTA |
| 951 | TTGGGTAATG | AATATCCGGT | GCTTGTCAAG | ATTACTCTCG | ACGAAGGTCA |
| | AACCCATTAC | TTATAGGCCA | CGAACAGTTC | TAATGAGAGC | TGCTTCCAGT |
| 1001 | GCCAGCGTAT | GCGCCTGGTC | TGTACACCGT | GCATCTGTCC | TCGTTCAAAG |
| | CGGTCGCATA | CGCGGACCAG | ACATGTGGCA | CGTAGACAGG | AGCAAGTTTC |
| 1051 | TTGGTCAGTT | CGGTTCTCTT | ATGATTGACC | GTCTGCGCCT | CGTTCCGGCT |
| | AACCAGTCAA | GCCAAGAGAA | TACTAACTGG | CAGACGCGGA | GCAAGGCCGA |
| 1101 | AAGTAACATG | GAGCAGGTCTG | CGGATTTCTGA | CACAATTTAT | CAGGCGATGA |
| | TTCAATTGTAC | CTCGTCCAGC | GCCTAAAGCT | GTGTAAATA | GTCCGCTACT |
| 1151 | TACAAATCTC | CGTTGTACTT | TGTTTCGCGC | TTGGTATAAT | CGCTGGGGGT |
| | ATGTTTAGAG | GCAACATGAA | ACAAAGCGCG | AACCATATTA | GCGACCCCCA |
| 1201 | CAAAGATGAG | TGTTTTAGTG | TATTCTTTCTG | CCTCTTTCTG | TTTAGGTTGG |
| | GTTTCTACTC | ACAAAATCAC | ATAAGAAAGC | GGAGAAAAGCA | AAATCCAACC |
| 1251 | TGCCTTCGTA | GTGGCATTAC | GTATTTTACC | CGTTTAAATGG | AAACTTCCTC |
| | ACGGAAGCAT | CACCGTAATG | CATAAAATGG | GCAAATTACC | TTTGAAGGAG |
| 1301 | ATGCGTAAGT | CTTTAGTCCT | CAAAGCCTCC | GTAGCCGTTG | CTACCCTCGT |
| | TACGCATTCA | GAAATCAGGA | GTTTCGGAGG | CATCGGCAAC | GATGGGAGCA |
| 1351 | TCCGATGCTG | TCTTTCGCTG | CTGAGGGTGA | CGATCCCGCA | AAAGCGGCCT |
| | AGGCTACGAC | AGAAAGCGAC | GACTCCCACT | GCTAGGGCGT | TTTCGCCGGA |
| 1401 | TTGACTCCCT | GCAAGCCTCA | GCGACCGAAT | ATATCGGTTA | TGCGTGGGCG |
| | AACTGAGGGA | CGTTCGGAGT | CGCTGGCTTA | TATAGCCAAT | ACGCACCCGC |
| 1451 | ATGGTTGTTG | TCATTGTCGG | CGCAACTATC | GGTATCAAGC | TGTTTAAGAA |
| | TACCAACAAC | AGTAACAGCC | GCGTTGATAG | CCATAGTTCG | ACAAATTCTT |
| 1501 | ATTCACCTCG | AAAGCAAGCT | GATAAAGGAG | GTTTCTCGAT | CGAGACGTTN |
| | TAAGTGGAGC | TTTCGTTCTGA | CTATTTCCTC | CAAAGAGCTA | GCTCTGCAAN |
| 1551 | NNNGAGGTTC | CAACTTTCAC | CATAATGAAA | TAAGATCACT | ACCGGGCGTA |
| | NNNCTCCAAG | GTTGAAAGTG | GTATTACTTT | ATTCTAGTGA | TGGCCCGCAT |
| 1601 | TTTTTTGAGT | TATCGAGATT | TTCAGGAGCT | AAGGAAGCTA | AAATGGAGAA |
| | AAAAAACTCA | ATAGCTCTAA | AAGTCCTCGA | TTCCTTCGAT | TTTACCTCTT |
| 1651 | AAAAATCACT | GGATATACCA | CCGTTGATAT | ATCCCAATGG | CATCGTAAAG |
| | TTTTTAGTGA | CCTATATGGT | GGCAACTATA | TAGGGTTACC | GTAGCATTTT |

6/39

1701 AACATTTTGA GGCATTTTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC
 TTGTAAACT CCGTAAAGTC AGTCAACGAG TTACATGGAT ATTGGTCTGG

1751 GTTCAGCTGG ATATTACGGC CTTTTTAAAG ACCGTAAAGA AAAATAAGCA
 CAAGTCGACC TATAATGCCG GAAAAATTTT TGGCATTCT TTTTATTCGT

1801 CAAGTTTTAT CCGGCCTTTA TTCACATTCT TGCCCGCCTG ATGAATGCTC
 GTTCAAAATA GGCCGGAAT AAGTGTAAGA ACGGGCGGAC TACTTACGAG

1851 ATCCGGAGTT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT
 TAGGCCTCAA GGCATACCGT TACTTTCTGC CACTCGACCA CTATACCTA

1901 AGTGTTACAC CTTGTTACAC CGTTTTCCAT GAGCAAACCTG AAACGTTTTTC
 TCACAAGTGG GAACAATGTG GCAAAAGGTA CTCGTTTGAC TTTGCAAAAG

1951 ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT
 TAGCGAGACC TCACTTATGG TGCTGCTAAA GGCCGTCAA GATGTGTATA

2001 ATTCGCAAGA TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA
 TAAGCGTTCT ACACCGCACA ATGCCACTTT TGGACCGGAT AAAGGGATTT

2051 GGGTTTATTG AGAATATGTT TTTCGTCTCA GCCAATCCCT GGGTGAGTTT
 CCCAAATAAC TCTTATACAA AAAGCAGAGT CGGTTAGGGA CCCACTCAAA

2101 CACCAGTTTT GATTTAAACG TGGCCAATAT GGACAACTTC TTCGCCCCCG
 GTGGTCAAAA CTAAATTTGC ACCGGTTATA CCTGTTGAAG AAGCGGGGGC

NcoI

~~~~~

2151 TTTTCACCAT GGGCAAAATAT TATACGCAAG GCGACAAGGT GCTGATGCCG  
 AAAAGTGGTA CCCGTTTATA ATATGCGTTC CGCTGTTCCA CGACTACGGC

2201 CTGGCGATTC AGGTTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG  
 GACCGCTAAG TCCAAGTAGT ACGGCAGACA CTACCGAAGG TACAGCCGTC

2251 AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGGCAG GGCGGGGCGT  
 TTACGAATTA CTTAATGTTG TCATGACGCT ACTCACCCTC CCGCCCCGCA

2301 AATTTTTTTA AGGCAGTTAT TGGTGCCCTT AAACGCCTGG TGCTACGCCT  
 TTAAAAAAT TCCGTCAATA ACCACGGGAA TTTGCGGACC ACGATGCGGA

2351 GAATAAGTGA TAATAAGCGG ATGAATGGCA GAAATTCGAA AGCAAATTCG  
 CTTATTCACT ATTATTTCGCC TACTTACCGT CTTTAAGCTT TCGTTTAAGC

2401 ACCCGGTCGT CGGTTTCAGGG CAGGGTCGTT AAATAGCCGC TTATGTCTAT  
 TGGGCCAGCA GCCAAGTCCC GTCCCAGCAA TTTATCGGCG AATACAGATA

2451 TGCTGGTTTA CCGGTTTATT GACTACCGGA AGCAGTGTGA CCGTGTGCTT  
 ACGACCAAAT GGCCAAATAA CTGATGGCCT TCGTCACACT GGCACACGAA

2501 CTCAAATGCC TGAGGCCAGT TTGCTCAGGC TCTCCCCGTG GAGGTAATAA  
 GAGTTTACGG ACTCCGGTCA AACGAGTCCG AGAGGGGCAC CTCCATTATT

7/39

2551 TTGCTCGACC GATAAAAGCG GCTTCCTGAC AGGAGGCCGT TTTGTTTTGC  
 AACGAGCTGG CTATTTTCGC CGAAGGACTG TCCTCCGGCA AAACAAAACG  
 2601 AGCCACCTC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC  
 TCGGGTGGAG TTGCGTTAAT TACTCTCAAT CGAGTGAGTA ATCCGTGGGG  
 2651 AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTGTGTGG AATTGTGAGC  
 TCCGAAATGT GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG  
 2701 GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTTCT  
 CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAAAGA  
 2751 AGATAACGAG GGCAAATCAT GAAAAAGACA GCTATCGCGA TTGCAGTGGC  
 TCTATTGCTC CCGTTTAGTA CTTTTTCTGT CGATAGCGCT AACGTCACCG  
 2801 ACTGGCTGGT TTCGCTACCG TAGCGCAGGC CGACTACAAA GATATCGTTA  
 TGACCGACCA AAGCGATGGC ATCGCGTCCG GCTGATGTTT CTATAGCAAT  
 2851 TGACCCAGTC ACCGTCCTCC CTGACCGTTA CCGCTGGTGA AAAAGTTACC  
 ACTGGGTCAG TGGCAGGAGG GACTGGCAAT GGCGACCACT TTTTCAATGG  
 2901 ATGTCCTGCA CCTCCTCCCA GTCCCTGTTC AACTCCGGTA AACAGAAAAA  
 TACAGGACGT GGAGGAGGGT CAGGGACAAG TTGAGGCCAT TTGTCTTTTT  
 2951 CTACCTGACC TGGTATCAGC AGAAACCGGG TCAGCCACCG AAAGTTCTGA  
 GATGGACTGG ACCATAGTCG TCTTTGGCCC AGTCGGTGGC TTTCAAGACT  
 3001 TCTACTGGGC TTCCACCCGT GAATCCGGTG TTCCAGACCG TTTCACCGGT  
 AGATGACCCG AAGGTGGGCA CTTAGGCCAC AAGGTCTGGC AAAGTGGCCA  
 3051 TCCGTTCCG GCACCGACTT CACCCTGACC ATCTCCTCCG TTCAGGCTGA  
 AGGCCAAGGC CGTGGCTGAA GTGGGACTGG TAGAGGAGGC AAGTCCGACT  
 3101 AGACCTGGCT GTTTACTACT GCCAGAACGA CTA CTCCAAC CCACTGACCT  
 TCTGGACCGA CAAATGATGA CGGTCTTGCT GATGAGGTTG GGTGACTGGA  
 3151 TCGGTGGTGG CACCAAACCTG GAACTTAAGC GCGCTGGTGG TGGAGGGTCT  
 AGCCACCACC GTGGTTTGAC CTTGAATTCTG CGCGACCACC ACCTCCCAGA

BamHI

~~~~~

3201 GGAGGAGGTG GGAGTGGGGG AGGTGGATCC GGCGGGGGAG GTTCAGGGGG
 CCTCCTCCAC CCTCACCCCC TCCACCTAGG CCGCCCCCTC CAAGTCCCCC
 3251 TGGCGGTAGT GGAGGGGGCG GTTCAGAAGT TCAACTAGTT GAATCCGGTG
 ACCGCCATCA CCTCCCCCGC CAAGTCTTCA AGTTGATCAA CTTAGGCCAC
 3301 GTGACCTGGT TAAACCGGGT GGTTCCCTGA AACTGTCCTG CGCTGCTTCC
 CACTGGACCA ATTTGGCCCA CCAAGGGACT TTGACAGGAC GCGACGAAGG

8/39

3351 GGTTCCTCCT TCTCCTCCTA CGGTATGTCC TGGGTTCGTC AGACCCCGGA
 CCAAAGAGGA AGAGGAGGAT GCCATACAGG ACCCAAGCAG TCTGGGGCCT

3401 CAAACGTCTG GAATGGGTTG CTACCATCTC CAACGGTGGT GGTACACCT
 GTTTGCAGAC CTTACCCAAC GATGGTAGAG GTTGCCACCA CCAATGTGGA

3451 ACTACCCGGA CTCCGTTAAA GGTCGTTTCA CCATCTCCCG TGACAACGCT
 TGATGGGCCT GAGGCAATTT CCAGCAAAGT GGTAGAGGGC ACTGTTGCGA

PstI

~~~~~

3501 AAAAACACCC TGTACCTGCA GATGTCCTCC CTGAAATCCG AAGACTCAGC  
 TTTTGTGGG ACATGGACGT CTACAGGAGG GACTTTAGGC TTCTGAGTCG

3551 TATGTACTAC TGCCTCGTC GTGAACGTTA CGACGAAAAC GGTTTCGCTT  
 ATACATGATG ACGCGAGCAG CACTTGCAAT GCTGCTTTTG CCAAAGCGAA

EcoRI

~~~~~

3601 ACTGGGGTCA GGGTACCCTG GTTACCGTTT CAGCTTCCGG AGAATTGAGC
 TGACCCAGT CCCATGGGAC CAATGGCAA GTCGAAGGCC TCTTAAGCTC

AvaI

~~~~~

3651 GCCTCGGGGG CCGAGGGCGG CGGTTCTGGT TCCGGTGATT TTGATTATGA  
 CGGAGCCCCC GGCTCCCGCC GCCAAGACCA AGGCCACTAA AACTAATACT

3701 AAAAATGGCA AACGCTAATA AGGGGGCTAT GACCGAAAAT GCCGATGAAA  
 TTTTACCCTG TTGCGATTAT TCCCCGATA CTGGCTTTTA CGGCTACTTT

3751 ACGCGCTACA GTCTGACGCT AAAGGCAAAC TTGATTCTGT CGCTACTGAT  
 TGCGCGATGT CAGACTGCGA TTTCCGTTTG AACTAAGACA GCGATGACTA

ClaI

~~~~~

3801 TACGGTGCTG CTATCGATGG TTTCATTGGT GACGTTTCCG GCCTTGCTAA
 ATGCCACGAC GATAGCTACC AAAGTAACCA CTGCAAAGGC CGGAACGATT

3851 TGGTAATGGT GCTACTGGTG ATTTTGCTGG CTCTAATTCC CAAATGGCTC
 ACCATTACCA CGATGACCAC TAAACGACC GAGATTAAGG GTTTACCGAG

3901 AAGTCGGTGA CGGTGATAAT TCACCTTTAA TGAATAATTT CCGTCAATAT
 TTCAGCCACT GCCACTATTA AGTGGAATTT ACTTATTAAA GGCAGTTATA

3951 TTACCTTCCC TCCCTCAATC GGTTGAATGT CGCCCTTTTG TCTTTGGCGC
 AATGGAAGGG AGGGAGTTAG CCAACTTACA GCGGGAAAAC AGAAACCGCG

4001 TGGTAAACCA TATGAATTTT CTATTGATTG TGACAAAATA AACTTATTCC
 ACCATTGGT AACTTAAAA GATAACTAAC ACTGTTTTAT TTGAATAAGG

4051 GTGGTGTCTT TGCCTTTCTT TTATATGTTG CCACCTTTAT GTATGTATTT
 CACCACAGAA ACGCAAAGAA AATATACAAC GGTGGAAATA CATACATAAA

9/39

HindIII

4101 TCTACGTTTG CTAACATACT GCGTAATAAG GAGTCTTGAT AAGCTTCGAG
AGATGCAAAC GATTGTATGA CGCATTATTC CTCAGAACTA TTCGAAGCTC

4151 AAATTCACCT CGAAAGCAAG CTGATAAACC GATACAATTA AAGGCTCCTT
TTTAAGTGGA GCTTTCGTTC GACTATTTGG CTATGTTAAT TTCCGAGGAA

EcoRI

4201 TTGGAGCCTT TTTTTTTGGA GAATTCAATC ATGCCAGTTC TTTTGGGTAT
AACCTCGGAA AAAAAAACCT CTTAAGTTAG TACGGTCAAG AAAACCCATA

4251 TCCGTTATTA TTGCGTTTCC TCGGTTTCCT TCTGGTAACT TTGTTCGGCT
AGGCAATAAT AACGCAAAGG AGCCAAAGGA AGACCATTGA AACAAGCCGA

4301 ATCTGCTTAC TTTCCTTAAA AAGGGCTTCG GTAAGATAGC TATTGCTATT
TAGACGAATG AAAGGAATTT TTCCCGAAGC CATTCTATCG ATAACGATAA

4351 TCATTGTTTC TTGCTCTTAT TATTGGGCTT AACTCAATTC TTGTGGGTTA
AGTAACAAAG AACGAGAATA ATAACCCGAA TTGAGTTAAG AACACCCAAT

4401 TCTCTCTGAT ATTAGCGCAC AATTACCCTC TGATTTTGTT CAGGGCGTTC
AGAGAGACTA TAATCGCGTG TTAATGGGAG ACTAAAACAA GTCCCGCAAG

4451 AGTTAATTCT CCCGTCTAAT GCGCTTCCCT GTTTTTATGT TATTCTCTCT
TCAATTAAGA GGGCAGATTA CGCGAAGGGA CAAAAATACA ATAAGAGAGA

4501 GTAAAGGCTG CTATTTTCAT TTTTGACGTT AAACAAAAAA TCGTTTCTTA
CATTTCCGAC GATAAAAGTA AAAACTGCAA TTTGTTTTTT AGCAAAGAAT

4551 TTTGGATTGG GATAAATAAA TATGGCTGTT TATTTTGTA CTGGCAAATT
AAACCTAACC CTATTTATTT ATACCGACAA ATAAAACATT GACCGTTTAA

4601 AGGCTCTGGA AAGACGCTCG TTAGCGTTGG TAAGATTCAG GATAAAATTG
TCCGAGACCT TTCTGCGAGC AATCGCAACC ATTCTAAGTC CTATTTTAAC

4651 TAGCTGGGTG CAAAATAGCA ACTAATCTTG ATTTAAGGCT TCAAAACCTC
ATCGACCCAC GTTTTATCGT TGATTAGAAC TAAATTCCGA AGTTTGGAG

4701 CCGCAAGTCG GGAGGTTTCG TAAACGCCT CGCGTTCTTA GAATACCGGA
GGCGTTCAGC CCTCCAAGCG ATTTTGCGGA GCGCAAGAAT CTTATGGCCT

4751 TAAGCCTTCT ATTTCTGATT TGCTTGCTAT TGGTCGTGGT AATGATTCCT
ATTCGGAAGA TAAAGACTAA ACGAACGATA ACCAGCACCA TTACTAAGGA

4801 ACGACGAAAA TAAAAACGGT TTGCTTGTTT TTGATGAATG CGGTACTTGG
TGCTGCTTTT ATTTTGGCCA AACGAACAAG AACTACTTAC GCCATGAACC

4851 TTTAATACCC GTTCATGGAA TGACAAGGAA AGACAGCCGA TTATTGATTG
AAATTATGGG CAAGTACCTT ACTGTTCCCT TCTGTCGGCT AATAACTAAC

10/39

| | | | | | |
|------|-------------|-------------|------------|------------|-------------|
| 4901 | GTTTCTTCAT | GCTCGTAAAT | TGGGATGGGA | TATTATTTTT | CTTGTTTCAGG |
| | CAAAGAAGTA | CGAGCATTTA | ACCCTACCTT | ATAATAAAAA | GAACAAGTCC |
| 4951 | ATTTATCTAT | TGTTGATAAA | CAGGCGCGTT | CTGCATTAGC | TGAACACGTT |
| | TAAATAGATA | ACAACTATTT | GTCCGCGCAA | GACGTAATCG | ACTTGTGCAA |
| 5001 | GTTTATTGTC | GCCGTCTGGA | CAGAATTACT | TTACCCTTTG | TCGGCACTTT |
| | CAAATAACAG | CGGCAGACCT | GTCTTAATGA | AATGGGAAAC | AGCCGTGAAA |
| 5051 | ATATTCTCTT | GTTACTGGCT | CAAAAATGCC | TCTGCCTAAA | TTACATGTTG |
| | TATAAGAGAA | CAATGACCGA | GTTTTTACGG | AGACGGATTT | AATGTACAAC |
| 5101 | GTGTTGTAA | ATATGGTGAT | TCTCAATTAA | GCCCTACTGT | TGAGCGTTGG |
| | CACAACAATT | TATAACCACTA | AGAGTTAATT | CGGGATGACA | ACTCGCAACC |
| 5151 | CTTTATACTG | GTAAGAATTT | ATATAACGCA | TATGACACTA | AACAGGCTTT |
| | GAAATATGAC | CATTCTTAAA | TATATTGCGT | ATACTGTGAT | TTGTCCGAAA |
| 5201 | TTCCAGTAAT | TATGATTCAG | GTGTTTATTC | ATATTTAACC | CCTTATTTAT |
| | AAGGTCATTA | ATACTAAGTC | CACAAATAAG | TATAAATTGG | GGAATAAATA |
| 5251 | CACACGGTCG | GTATTTCAAA | CCATTAAATT | TAGGTCAGAA | GATGAAATTA |
| | GTGTGCCAGC | CATAAAGTTT | GGTAATTTAA | ATCCAGTCTT | CTACTTTAAT |
| 5301 | ACTAAAATAT | ATTTGAAAAA | GTTTTCTCGC | GTTCTTTGTC | TTGCGATAGG |
| | TGATTTTATA | TAAACTTTTT | CAAAAGAGCG | CAAGAAACAG | AACGCTATCC |
| 5351 | ATTTGCATCA | GCATTTACAT | ATAGTTATAT | AACCCAACCT | AAGCCGGAGG |
| | TAAACGTAGT | CGTAAATGTA | TATCAATATA | TTGGGTTGGA | TTCGGCCTCC |
| 5401 | TTAAAAAGGT | AGTCTCTCAG | ACCTATGATT | TTGATAAATT | CACTATTGAC |
| | AATTTTTCCTA | TCAGAGAGTC | TGGATACTAA | AACTATTTAA | GTGATAACTG |
| 5451 | TCTTCTCAGC | GTCTTAATCT | AAGCTATCGC | TATGTTTTCA | AGGATTCTAA |
| | AGAAGAGTCG | CAGAAATTAG | TTGATAGCG | ATACAAAAGT | TCCTAAGATT |
| 5501 | GGGAAAATTA | ATTAATAGCG | ACGATTTACA | GAAGCAAGGT | TATTCCATCA |
| | CCCTTTTAAT | TAATTATCGC | TGCTAAATGT | CTTCGTTCCA | ATAAGGTAGT |
| 5551 | CATATATTGA | TTTATGTACT | GTTTCAATTA | AAAAAGGTAA | TTCAAATGAA |
| | GTATATAACT | AAATACATGA | CAAAGTTAAT | TTTTTCCATT | AAGTTTACTT |
| 5601 | ATTGTTAAAT | GTAATTAATT | TTGTTTTCTT | GATGTTTGTT | TCATCATCTT |
| | TAACAATTTA | CATTAATTAA | AACAAAAGAA | CTACAAACAA | AGTAGTAGAA |
| 5651 | CTTTTGCTCA | AGTAATTGAA | ATGAATAATT | CGCCTCTGCG | CGATTTCTGTG |
| | GAAAACGAGT | TCATTAACCTT | TACTTATTAA | GCGGAGACGC | GCTAAAGCAC |
| 5701 | ACTTGGTATT | CAAAGCAAAC | AGGTGAATCT | GTTATTGTCT | CACCTGATGT |
| | TGAACCATAA | GTTTCGTTTG | TCCACTTAGA | CAATAACAGA | GTGGACTACA |

11/39

| | | | | | |
|------|------------|-------------|-------------|------------|------------|
| 5751 | TAAAGGTACA | GTGACTGTAT | ATTCCTCTGA | CGTTAAGCCT | GAAAATTTAC |
| | ATTTCCATGT | CACTGACATA | TAAGGAGACT | GCAATTCGGA | CTTTTAAATG |
| 5801 | GCAATTTCTT | TATCTCTGTT | TTACGTGCTA | ATAATTTTGA | TATGGTTGGC |
| | CGTTAAAGAA | ATAGAGACAA | AATGCACGAT | TATTAAAACT | ATACCAACCG |
| 5851 | TCAATTCCTT | CCATAATTCA | GAAATATAAC | CCAAATAGTC | AGGATTATAT |
| | AGTTAAGGAA | GGTATTAAGT | CTTTATATTG | GGTTTATCAG | TCCTAATATA |
| 5901 | TGATGAATTG | CCATCATCTG | ATATTCAGGA | ATATGATGAT | AATTCCGCTC |
| | ACTACTTAAC | GGTAGTAGAC | TATAAGTCCT | TATACTACTA | TTAAGGCGAG |
| 5951 | CTTCTGGTGG | TTTCTTTGTT | CCGCAAAATG | ATAATGTTAC | TCAAACATTT |
| | GAAGACCACC | AAAGAAACAA | GGCGTTTTTAC | TATTACAATG | AGTTTGTAAA |
| 6001 | AAAATTAATA | ACGTTTCGCGC | AAAGGATTTA | ATAAGGGTTG | TAGAATTGTT |
| | TTTTAATTAT | TGCAAGCGCG | TTTCCTAAAT | TATTCCCAAC | ATCTTAACAA |
| 6051 | TGTTAAATCT | AATACATCTA | AATCCTCAAA | TGTATTATCT | GTTGATGGTT |
| | ACAATTTAGA | TTATGTAGAT | TTAGGAGTTT | ACATAATAGA | CAACTACCAA |
| 6101 | CTAACTTATT | AGTAGTTAGC | GCCCCTAAAG | ATATTTTAGA | TAACCTTCCG |
| | GATTGAATAA | TCATCAATCG | CGGGGATTTC | TATAAAATCT | ATTGGAAGGC |
| 6151 | CAATTTCTTT | CTACTGTTGA | TTTGCCAACT | GACCAGATAT | TGATTGAAGG |
| | GTTAAAGAAA | GATGACAACT | AAACGGTTGA | CTGGTCTATA | ACTAACTTCC |
| 6201 | ATTAATTTTC | GAGGTTTCAGC | AAGGTGATGC | TTTAGATTTT | TCCTTTGCTG |
| | TAATTAAAAG | CTCCAAGTCG | TTCCACTACG | AAATCTAAAA | AGGAAACGAC |
| 6251 | CTGGCTCTCA | GCGCGGCACT | GTTGCTGGTG | GTGTTAATAC | TGACCGTCTA |
| | GACCGAGAGT | CGCGCCGTGA | CAACGACCAC | CACAATTATG | ACTGGCAGAT |
| 6301 | ACCTCTGTTT | TATCTTCTGC | GGGTGGTTTCG | TTCGGTATTT | TTAACGGCGA |
| | TGGAGACAAA | ATAGAAGACG | CCCACCAAGC | AAGCCATAAA | AATTGCCGCT |
| 6351 | TGTTTTAGGG | CTATCAGTTC | GCGCATTAAA | GACTAATAGC | CATTCAAAAA |
| | ACAAAATCCC | GATAGTCAAG | CGCGTAATTT | CTGATTATCG | GTAAGTTTTT |
| 6401 | TATTGTCTGT | GCCTCGTATT | CTTACGCTTT | CAGGTCAGAA | GGGTTCTATT |
| | ATAACAGACA | CGGAGCATAA | GAATGCGAAA | GTCCAGTCTT | CCCAAGATAA |
| 6451 | TCTGTTGGCC | AGAATGTCCC | TTTTATTACT | GGTCGTGTAA | CTGGTGAATC |
| | AGACAACCGG | TCTTACAGGG | AAAATAATGA | CCAGCACATT | GACCACTTAG |
| 6501 | TGCCAATGTA | AATAATCCAT | TTCAGACGGT | TGAGCGTCAA | AATGTTGGTA |
| | ACGGTTACAT | TTATTAGGTA | AAGTCTGCCA | ACTCGCAGTT | TTACAACCAT |
| 6551 | TTTCTATGAG | TGTTTTTCCC | GTTGCAATGG | CTGGCGGTAA | TATTGTTTTA |
| | AAAGATACTC | ACAAAAAGGG | CAACGTTACC | GACCGCCATT | ATAACAAAAT |

12/39

6601 GATATAACCA GTAAGGCCGA TAGTTTGAGT TCTTCTACTC AGGCAAGTGA
CTATATTGGT CATTCCGGCT ATCAAACCTCA AGAAGATGAG TCCGTTCACT

6651 TGTTATTACT AATCAAAGAA GTATTGCGAC AACGGTTAAT TTGCGTGATG
ACAATAATGA TTAGTTTCTT CATAACGCTG TTGCCAATTA AACGCACTAC

6701 GTCAGACTCT TTTGCTCGGT GGCCTCACTG ATTACAAAAA CACTTCTCAA
CAGTCTGAGA AAACGAGCCA CCGGAGTGAC TAATGTTTTT GTGAAGAGTT

6751 GATTCTGGTG TGCCGTTTCT GTCTAAAATC CCTTTAATCG GCCTCCTGTT
CTAAGACCAC ACGGCAAGGA CAGATTTTATG GGAAATTAGC CGGAGGACAA

6801 TAGCTCCCGT TCTGATTCTA ACGAGGAAAG CACGTTGTAC GTGCTCGTCA
ATCGAGGGCA AGACTAAGAT TGCTCCTTTC GTGCAACATG CACGAGCAGT

6851 AAGCAACCAT AGTACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT
TTCGTTGGTA TCATGCGCGG GACATCGCCG CGTAATTCGC GCCGCCACA

6901 GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG
CCACCAATGC GCGTCGCACT GCGGATGTGA ACGGTCGCGG GATCGCGGGC

6951 CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCTC CGGCTTTCCC
GAGGAAAGCG AAAGAAGGGA AGGAAAGAGC GGTGCAAGAG GCCGAAAGGG

BamHI

~~~~~

7001 CGTCAAGCTC TAAATCGGGG GATCCCTTTA GGGTTCCGAT TTAGTGCTTT  
GCAGTTCGAG ATTTAGCCCC CTAGGGAAAT CCCAAGGCTA AATCACGAAA

7051 ACGGCACCTC GACCTCCAAA AACTTGATTT GGGTGATGGT TCACGTAGTG  
TGCCGTGGAG CTGGAGGTTT TTGAACTAAA CCCACTACCA AGTGCATCAC

7101 GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG  
CCGGTAGCGG GACTATCTGC CAAAAAGCGG GAAACTGCAA CCTCAGGTGC

7151 TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCACAACTAA  
AAGAAATTAT CACCTGAGAA CAAGGTTTGA CTTGTTGTG AGTGTTGATT

7201 CTCGGCCTAT TCTTTTGATT TATAAGGATT TTTGTCATTT TCTGCTTACT  
GAGCCGGATA AGAAAACATA ATATTCCTAA AAACAGTAAA AGACGAATGA

7251 GGTTAAAAAA TAAGCTGATT TAACAAATAT TTAACGCGAA ATTTAACAAA  
CCAATTTTTT ATTCGACTAA ATTGTTTATA AATTGCGCTT TAAATTGTTT

7301 ACATTAACGT TTACAATTTA AATATTTGCT TATACAATCA TCCTGTTTTT  
TGTAATTGCA AATGTTAAAT TTATAAACGA ATATGTTAGT AGGACAAAAA

7351 GGGGCTTTTC TGATTATCAA CCGGGGTACA TATGATTGAC ATGCTAGTTT  
CCCCGAAAAG ACTAATAGTT GGCCCCATGT ATACTAAGT TACGATCAAA

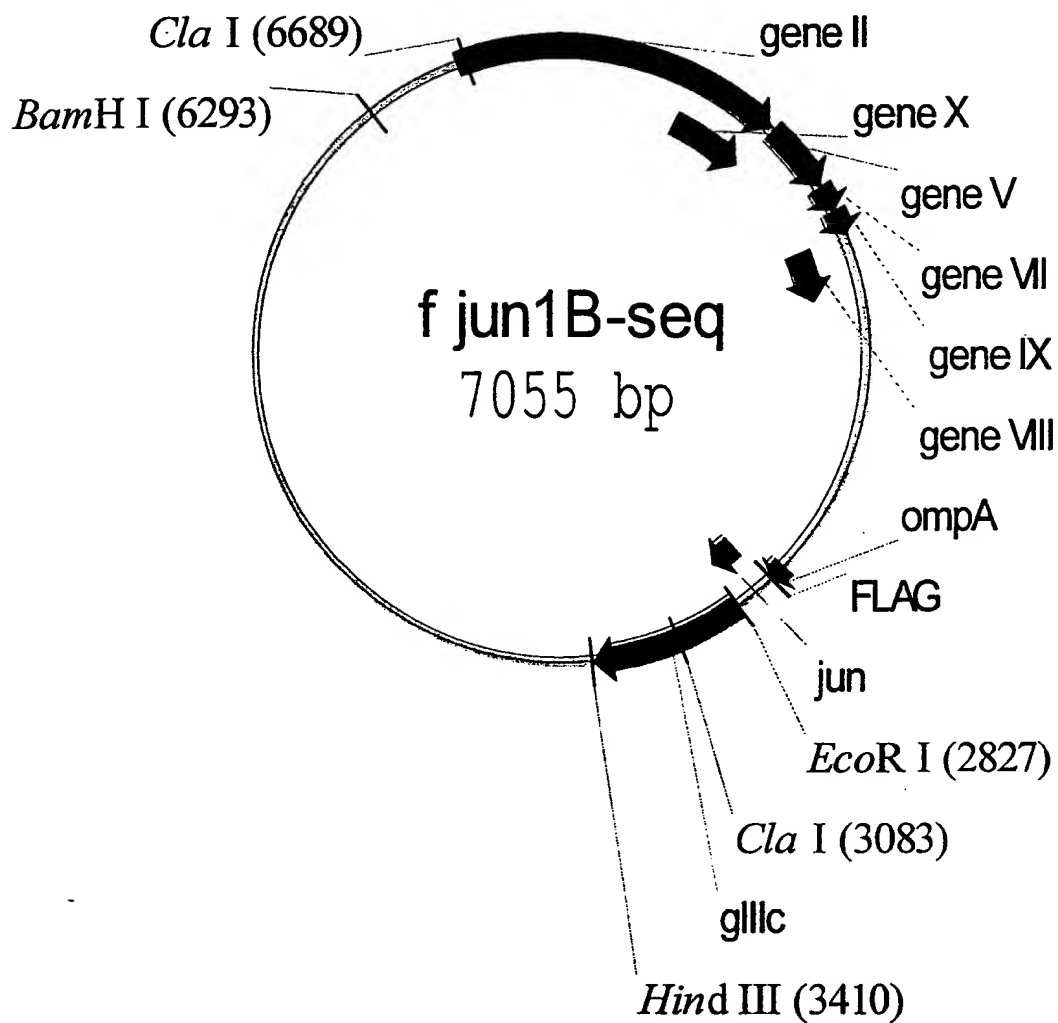
13/39

Clai

~~~~~

| | | | | | |
|------|------------|-------------|-------------|------------|------------|
| 7401 | TACGATTACC | GTTTCATCGAT | TCTCTTGTTT | GCTCCAGACT | TTCAGGTAAT |
| | ATGCTAATGG | CAAGTAGCTA | AGAGAACAAA | CGAGGTCTGA | AAGTCCATTA |
| 7451 | GACCTGATAG | CCTTTGTAGA | CCTCTCAAAA | ATAGCTACCC | TCTCCGGCAT |
| | CTGGACTATC | GGAAACATCT | GGAGAGTTTT | TATCGATGGG | AGAGGCCGTA |
| 7501 | GAATTTATCA | GCTAGAACGG | TTGAATATCA | TATTGACGGT | GATTTGACTG |
| | CTTAAATAGT | CGATCTTGCC | AAC TTATAGT | ATAACTGCCA | CTAAACTGAC |
| 7551 | TCTCCGGCCT | TTCTCACCCG | TTTGAATCTT | TGCCTACTCA | TTACTCCGGC |
| | AGAGGCCGGA | AAGAGTGGGC | AACTTAGAA | ACGGATGAGT | AATGAGGCCG |
| 7601 | ATTGCATTTA | AAATATATGA | GGGTTCTAAA | AATTTTATC | CCTGCGTTGA |
| | TAACGTAAAT | TTTATATACT | CCCAAGATTT | TTAAAAATAG | GGACGCAACT |
| 7651 | AATTAAGGCT | TCACCAGCAA | AAGTATTACA | GGGTCATAAT | GTTTTTGGTA |
| | TTAATTCCGA | AGTGGTCGTT | TTCATAATGT | CCCAGTATTA | CAAAAACCAT |
| 7701 | CAACCGATTT | AGCTTTATGC | TCTGAGGCTT | TATTGCTTAA | TTTTGCTAAC |
| | GTTGGCTAAA | TCGAAATACG | AGACTCCGAA | ATAACGAATT | AAAACGATTG |
| 7751 | TCTCTGCCTT | GCTTGACGA | TTTATTGGAT | GTT | |
| | AGAGACGGAA | CGAACATGCT | AAATAACCTA | CAA | |

14/39

Figure 3

15/39

| | | | | | |
|-----|------------|------------|------------|------------|-------------|
| 1 | AACGCTACTA | CCATTAGTAG | AATTGATGCC | ACCTTTTCAG | CTCGCGCCCC |
| | TTGCGATGAT | GGTAATCATC | TTAACTACGG | TGGAAAAGTC | GAGCGCGGGG |
| 51 | AAATGAAAAT | ATAGCTAAAC | AGGTTATTGA | CCATTTGCGA | AATGTATCTA |
| | TTTACTTTTA | TATCGATTG | TCCAATAACT | GGTAAACGCT | TTACATAGAT |
| 101 | ATGGTCAAAC | TAAATCTACT | CGTTCGCAGA | ATTGGGAATC | AACTGTTACA |
| | TACCAGTTTG | ATTTAGATGA | GCAAGCGTCT | TAACCCTTAG | TTGACAAATGT |
| 151 | TGGAATGAAA | CTTCCAGACA | CCGTACTTTA | GTTGCATATT | TAAAACATGT |
| | ACCTTACTTT | GAAGGTCTGT | GGCATGAAAT | CAACGTATAA | ATTTTGTACA |
| 201 | TGAACTACAG | CACCAGATTC | AGCAATTAAG | CTCTAAGCCA | TCCGCAAAAA |
| | ACTTGATGTC | GTGGTCTAAG | TCGTTAATTC | GAGATTCGGT | AGGCGTTTTT |
| 251 | TGACCTCTTA | TCAAAAGGAG | CAATTAAAGG | TACTGTCTAA | TCCTGACCTG |
| | ACTGGAGAAT | AGTTTTCTCT | GTTAATTTCC | ATGACAGATT | AGGACTGGAC |
| 301 | TTGGAATTTG | CTTCCGGTCT | GGTTCGCTTT | GAGGCTCGAA | TTGAAACGCG |
| | AACCTTAAAC | GAAGGCCAGA | CCAAGCGAAA | CTCCGAGCTT | AACTTTGCGC |
| 351 | ATATTTGAAG | TCTTTCGGGC | TTCCTCTTAA | TCTTTTTGAT | GCAATTCGCT |
| | TATAAACTTC | AGAAAGCCCC | AAGGAGAATT | AGAAAACTA | CGTTAAGCGA |
| 401 | TTGCTTCTGA | CTATAATAGA | CAGGGTAAAG | ACCTGATTTT | TGATTTATGG |
| | AACGAAGACT | GATATTATCT | GTCCCATTTT | TGGACTAAAA | ACTAAATACC |
| 451 | TCATTCTCGT | TTTCTGAACT | GTTTAAAGCA | TTTGAGGGGG | ATTCAATGAA |
| | AGTAAGAGCA | AAAGACTTGA | CAAATTTTCG | AAACTCCCCC | TAAGTTACTT |
| 501 | TATTTATGAC | GATTCCGCAG | TATTGGACGC | TATCCAGTCT | AAACATTTTA |
| | ATAAATACTG | CTAAGGCGTC | ATAACCTGCG | ATAGGTCAGA | TTTGTAAAAA |
| 551 | CAATTACCCC | CTCTGGCAAA | ACTTCCTTTG | CAAAAGCCTC | TCGCTATTTT |
| | GTTAATGGGG | GAGACCGTTT | TGAAGGAAAC | GTTTTCGGAG | AGCGATAAAA |
| 601 | GGTTTCTATC | GTCGTCTGGT | TAATGAGGGT | TATGATAGTG | TTGCTCTTAC |
| | CCAAAGATAG | CAGCAGACCA | ATTACTCCCA | ATACTATCAC | AACGAGAATG |
| 651 | CATGCCTCGT | AATTCCTTTT | GGCGTTATGT | ATCTGCATTA | GTTGAGTGTG |
| | GTACGGAGCA | TTAAGGAAAA | CCGCAATACA | TAGACGTAAT | CAACTCACAC |
| 701 | GTATTCCTAA | ATCTCAATTG | ATGAATCTTT | CCACCTGTAA | TAATGTTGTT |
| | CATAAGGATT | TAGAGTTAAC | TACTTAGAAA | GGTGGACATT | ATTACAACAA |
| 751 | CCGTTAGTTC | GTTTTATTAA | CGTAGATTTT | TCCTCCCAAC | GTCCTGACTG |
| | GGCAATCAAG | CAAAATAATT | GCATCTAAAA | AGGAGGGTTG | CAGGACTGAC |
| 801 | GTATAATGAG | CCAGTTCTTA | AAATCGCATA | AGGTAATTCA | AAATGATTAA |
| | CATATTACTC | GGTCAAGAAT | TTTAGCGTAT | TCCATTAAGT | TTTACTAATT |

16/39

| | | | | | |
|------|------------|-------------|-------------|-------------|-------------|
| 851 | AGTTGAAATT | AAACCGTCTC | AAGCGCAATT | TACTACCCGT | TCTGGTGTTC |
| | TCAACTTTAA | TTTGGCAGAG | TTGCGTTAA | ATGATGGGCA | AGACCACAAA |
| 901 | CTCGTCAGGG | CAAGCCTTAT | TCACTGAATG | AGCAGCTTTG | TTACGTTGAT |
| | GAGCAGTCCC | GTTCGGAATA | AGTGACTTAC | TCGTCGAAAC | AATGCAACTA |
| 951 | TTGGGTAATG | AATATCCGGT | GCTTGTCAAG | ATTACTCTCG | ACGAAGGTCA |
| | AACCCATTAC | TTATAGGCCA | CGAACAGTTC | TAATGAGAGC | TGCTTCCAGT |
| 1001 | GCCAGCGTAT | GCGCCTGGTC | TGTACACCGT | GCATCTGTCC | TCGTTCAAAG |
| | CGGTCGCATA | CGCGGACCAG | ACATGTGGCA | CGTAGACAGG | AGCAAAGTTTC |
| 1051 | TTGGTCAGTT | CGGTTCTCTT | ATGATTGACC | GTCTGCGCCT | CGTTCCGGCT |
| | AACCAGTCAA | GCCAAGAGAA | TACTAACTGG | CAGACGCGGA | GCAAGGCCGA |
| 1101 | AAGTAACATG | GAGCAGGTCTG | CGGATTTCTGA | CACAATTTAT | CAGGCGATGA |
| | TTCATTGTAC | CTCGTCCAGC | GCCTAAAGCT | GTGTTAAATA | GTCCGCTACT |
| 1151 | TACAAATCTC | CGTTGTACTT | TGTTTCGCGC | TTGGTATAAT | CGCTGGGGGT |
| | ATGTTTAGAG | GCAACATGAA | ACAAAGCGCG | AACCATATTA | GCGACCCCCA |
| 1201 | CAAAGATGAG | TGTTTTAGTG | TATTCTTTCTG | CCTCTTTCTG | TTTAGGTTGG |
| | GTTTCTACTC | ACAAAATCAC | ATAAGAAAGC | GGAGAAAGCA | AAATCCAACC |
| 1251 | TGCCTTCGTA | GTGGCATTAC | GTATTTTACC | CGTTTAATGG | AAACTTCCTC |
| | ACGGAAGCAT | CACCGTAATG | CATAAAATGG | GCAAATTACC | TTTGAAGGAG |
| 1301 | ATGCGTAAGT | CTTTAGTCCT | CAAAGCCTCC | GTAGCCGTTG | CTACCCCTCGT |
| | TACGCATTCA | GAAATCAGGA | GTTTCGGAGG | CATCGGCAAC | GATGGGAGCA |
| 1351 | TCCGATGCTG | TCTTTCGCTG | CTGAGGGTGA | CGATCCCGCA | AAAGCGGCCT |
| | AGGCTACGAC | AGAAAGCGAC | GACTCCCACT | GCTAGGGCGT | TTTCGCCGGA |
| 1401 | TTGACTCCCT | GCAAGCCTCA | GCGACCGAAT | ATATCGGTTA | TGCGTGGGCG |
| | AACTGAGGGA | CGTTCGGAGT | CGCTGGCTTA | TATAGCCAAT | ACGCACCCGC |
| 1451 | ATGGTTGTTG | TCATTGTCGG | CGCAACTATC | GGTATCAAGC | TGTTTAAGAA |
| | TACCAACAAC | AGTAACAGCC | GCGTTGATAG | CCATAGTTCTG | ACAAATTCTT |
| 1501 | ATTACCTCTG | AAAGCAAGCT | GATAAAGGAG | GTTTCTCGAT | CGAGACGTTN |
| | TAAGTGGAGC | TTTCGTTCTGA | CTATTTCTCTC | CAAAGAGCTA | GCTCTGCAAN |
| 1551 | NNNGAGGTTC | CAACTTTCAC | CATAATGAAA | TAAGATCACT | ACCGGGCGTA |
| | NNNCTCCAAG | GTTGAAAGTG | GTATTACTTT | ATTCTAGTGA | TGGCCCCGAT |
| 1601 | TTTTTTGAGT | TATCGAGATT | TTCAGGAGCT | AAGGAAGCTA | AAATGGAGAA |
| | AAAAAATCA | ATAGCTCTAA | AAGTCCTCGA | TTCCTTCGAT | TTTACCTCTT |
| 1651 | AAAAATCACT | GGATATACCA | CCGTTGATAT | ATCCCAATGG | CATCGTAAAG |
| | TTTTTAGTGA | CCTATATGGT | GGCAACTATA | TAGGGTTACC | GTAGCATTTT |

17/39

| | | | | | |
|------|-------------|-------------|-------------|-------------|-------------|
| 1701 | AACATTTTGA | GGCATTTTCAG | TCAGTTGCTC | AATGTACCTA | TAACCAGACC |
| | TTGTAAAACT | CCGTAAAGTC | AGTCAACGAG | TTACATGGAT | ATTGGTCTGG |
| 1751 | G TTCAGCTGG | ATATTACGGC | CTTTTTTAAAG | ACCGTAAAGA | AAAAATAAGCA |
| | CAAGTCGACC | TATAATGCCG | GAAAAATTTT | TGGCATTTCT | TTTTATTTCGT |
| 1801 | CAAGTTTTAT | CCGGCCTTTA | TTCACATTCT | TGCCCCGCTG | ATGAATGCTC |
| | GTTCAAAATA | GGCCGGAAAT | AAGTGTAAGA | ACGGGCGGAC | TACTTACGAG |
| 1851 | ATCCGGAGTT | CCGTATGGCA | ATGAAAGACG | GTGAGCTGGT | GATATGGGAT |
| | TAGGCCTCAA | GGCATAACCGT | TACTTTCTGC | CACTCGACCA | CTATACCCTA |
| 1901 | AGTGTTTACC | CTTGTTACAC | CGTTTTTCCAT | GAGCAAACCTG | AAACGTTTTTC |
| | TCACAAGTGG | GAACAATGTG | GCAAAAAGGTA | CTCGTTTGAC | TTTGCAAAAG |
| 1951 | ATCGCTCTGG | AGTGAATACC | ACGACGATTT | CCGGCAGTTT | CTACACATAT |
| | TAGCGAGACC | TCACTTATGG | TGCTGCTAAA | GGCCGTCAAA | GATGTGTATA |
| 2001 | ATTTCGAAGA | TGTGGCGTGT | TACGGTGAAA | ACCTGGCCTA | TTTCCCTAAA |
| | TAAGCGTTCT | ACACCGCACA | ATGCCACTTT | TGGACCGGAT | AAAGGGATTT |
| 2051 | GGGTTTATTG | AGAATATGTT | TTTCGTCTCA | GCCAATCCCT | GGGTGAGTTT |
| | CCCAAATAAC | TCTTATACAA | AAAGCAGAGT | CGGTTAGGGA | CCCACTCAAA |
| 2101 | CACCAGTTTT | GATTTAAACG | TAGCCAATAT | GGACAACTTC | TTCGCCCCCG |
| | GTGGTCAAAA | CTAAATTTGC | ATCGGTTATA | CCTGTTGAAG | AAGCGGGGGC |
| 2151 | TTTTCACTAT | GGGCAAATAT | TATACGCAAG | GCGACAAGGT | GCTGATGCCG |
| | AAAAGTGATA | CCCGTTTATA | ATATGCGTTC | CGCTGTTCCA | CGACTACGGC |
| 2201 | CTGGCGATT | AGGTTTATCA | TGCCGTTTGT | GATGGCTTCC | ATGTCGGCAG |
| | GACCGCTAAG | TCCAAGTAGT | ACGGCAAACA | CTACCGAAGG | TACAGCCGTC |
| 2251 | AATGCTTAAT | GAATTACAAC | AGTACTGCGA | TGAGTGGCAG | GGCGGGGCGT |
| | TTACGAATTA | CTTAATGTTG | TCATGACGCT | ACTCACCGTC | CCGCCCCGCA |
| 2301 | AATTTTTTTA | AGGCAGTTAT | TGGTGCCCTT | AAACGCCTGG | TGCTAGCCTG |
| | TTAAAAAAAT | TCCGTCAATA | ACCACGGGAA | TTTGCGGACC | ACGATCGGAC |
| 2351 | AGGCCAGTTT | GCTCAGGCTC | TCCCCGTGGA | GGTAATAATT | GCTCGACCGA |
| | TCCGGTCAAA | CGAGTCCGAG | AGGGGCACCT | CCATTATTAA | CGAGCTGGCT |
| 2401 | TAAAAGCGGC | TTCTTGACAG | GAGGCCGTTT | TGTTTTGCAG | CCCACCTCAA |
| | ATTTTCGCCG | AAGGACTGTC | CTCCGGCAAA | ACAAAACGTC | GGGTGGAGTT |
| 2451 | CGCAATTAAT | GTGAGTTAGC | TCACTCATTA | GGCACCCAG | GCTTTTACACT |
| | GCGTTAATTA | CACTCAATCG | AGTGAGTAAT | CCGTGGGGTC | CGAAATGTGA |
| 2501 | TTATGCTTCC | GGCTCGTATG | TTGTGTGGAA | TTGTGAGCGG | ATAACAATTT |
| | AATACGAAGG | CCGAGCATAC | AACACACCTT | AACACTCGCC | TATTGTAAAA |

18/39

2551 CACACAGGAA ACAGCTATGA CCATGATTAC GAATTTCTAG ATAACGAGGG
GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAAAGATC TATTGCTCCC

2601 CAAAAAATGA AAAAGACAGC TATCGCGATT GCAGTGGCAC TGGCTGGTTT
GTTTTTTTACT TTTTCTGTCTG ATAGCGCTAA CGTCACCGTG ACCGACCAAA

2651 CGCTACCGTA GCGCAGGCCG ACTACAAAGA TGTCGACGCC GGTGGTCGGA
GCGATGGCAT CGCGTCCGGC TGATGTTTCT ACAGCTGCGG CCACCAGCCT

2701 TCGCCCGGCT AGAGGAAAAA GTGAAAACCT TGAAAGCGCA AAACCTCCGAG
AGCGGGCCGA TCTCCTTTTT CACTTTTGA AACTTCGCGT TTTGAGGCTC

2751 CTGGCGTCCA CGGCCAACAT GCTCAGGGAA CAGGTGGCAC AGCTTAAACA
GACCGCAGGT GCCGGTTGTA CGAGTCCCTT GTCCACCGTG TCGAATTTGT

EcoRI

~~~~~

2801 GAAAGTCATG AACCACGGTG GTGCCGAATT CAATGCTGGC GGCGGCTCTG  
CTTTCAGTAC TTGGTGCCAC CACGGCTTAA GTTACGACCG CCGCCGAGAC

2851 GTGGTGGTTC TGGTGGCGGC TCTGAGGGTG GTGGCTCTGA GGGTGGCGGT  
CACCACCAAG ACCACCGCCG AGACTCCAC CACCGAGACT CCCACCGCCA

2901 TCTGAGGGTG GCGGCTCTGA GGGAGGCGGT TCCGGTGGTG GCTCTGGTTC  
AGACTCCAC CGCCGAGACT CCCTCCGCCA AGGCCACCAC CGAGACCAAG

2951 CGGTGATTTT GATTATGAAA AGATGGCAAA CGCTAATAAG GGGGCTATGA  
GCCACTAAAA CTAATACTTT TCTACCGTTT GCGATTATTC CCCCATACT

3001 CCGAAAATGC CGATGAAAAC GCGCTACAGT CTGACGCTAA AGGCAAACCTT  
GGCTTTTACG GCTACTTTTG CGCGATGTCA GACTGCGATT TCCGTTTGAA

ClaI

~~~~~

3051 GATTCTGTCTG CTAATGATTA CGGTGCTGCT ATCGATGGTT TCATTGGTGA
CTAAGACAGC GATGACTAAT GCCACGACGA TAGCTACCAA AGTAACCACT

3101 CGTTTCCGGC CTTGCTAATG GTAATGGTGC TACTGGTGAT TTTGCTGGCT
GCAAAGGCCG GAACGATTAC CATTACCACG ATGACCACTA AAACGACCGA

3151 CTAATCCCA AATGGCTCAA GTCGGTGACG GTGATAATTC ACCTTTAATG
GATTAAGGGT TTACCGAGTT CAGCCACTGC CACTATTAAG TGGAAATTAC

3201 AATAATTTCC GTCAATATTT ACCTTCCCTC CCTCAATCGG TTGAATGTCTG
TTATTAAAGG CAGTTATAAA TGGAAGGGAG GGAGTTAGCC AACTTACAGC

3251 CCCTTTTGTC TTTAGCGCTG GTAAACCATA TGAATTTTCT ATTGATTGTG
GGGAAAACAG AAATCGCGAC CATTTGGTAT ACTTAAAAGA TAACTAACAC

3301 ACAAATAAAA CTTATTCCGT GGTGTCTTTG CGTTTCTTTT ATATGTTGCC
TGTTTTATTT GAATAAGGCA CCACAGAAAC GCAAAGAAAA TATACAACGG

19/39

3351 ACCTTTATGT ATGTATTTTC TACGTTTGCT AACATACTGC GTAATAAGGA
TGGAAATACA TACATAAAAG ATGCAAACGA TTGTATGACG CATTATTCCT

HindIII

~~~~~

3401 GTCTTGATAA GCTTCGAGAA ATTCACCTCG AAAGCAAGCT GATAAACCGA  
CAGAACTATT CGAAGCTCTT TAAGTGGAGC TTTCGTTCTGA CTATTTGGCT

3451 TACAATTAAA GGCTCCTTTT GGAGCCTTTT TTTTGGAGA ATTAATTCAA  
ATGTTAATTT CCGAGGAAAA CCTCGGAAAA AAAACCTCT TAATTAAGTT

3501 TCATGCCAGT TCTTTTGGGT ATTCCGTTAT TATTGCGTTT CCTCGGTTTC  
AGTACGGTCA AGAAAACCCA TAAGGCAATA ATAACGCAA GGAGCCAAAG

3551 CTTCTGGTAA CTTTGTTCGG CTATCTGCTT ACTTTCCTTA AAAAGGGCTT  
GAAGACCATT GAAACAAGCC GATAGACGAA TGAAAGGAAT TTTTCCCGAA

3601 CGGTAAGATA GCTATTGCTA TTTCATTGTT TCTTGCTCTT ATTATTGGGC  
GCCATTCTAT CGATAACGAT AAAGTAACAA AGAACGAGAA TAATAACCCG

3651 TTAACCTCAAT TCTTGTGGGT TATCTCTCTG ATATTAGCGC ACAATTACCC  
AATTGAGTTA AGAACACCCA ATAGAGAGAC TATAATCGCG TGTTAATGGG

3701 TCTGATTTTG TTCAGGGCGT TCAGTTAATT CTCCCGTCTA ATGCGCTTCC  
AGACTAAAC AAGTCCCGCA AGTCAATTAA GAGGGCAGAT TACGCGAAGG

3751 CTGTTTTTAT GTTATTCTCT CTGTAAAGGC TGCTATTTTC ATTTTGGACG  
GACAAAATA CAATAAGAGA GACATTTCCG ACGATAAAAG TAAAACTGC

3801 TTAAACAAAA AATCGTTTCT TATTTGGATT GGGATAAATA AATATGGCTG  
AATTTGTTTT TTAGCAAAGA ATAAACCTAA CCCTATTTAT TTATACCGAC

3851 TTTATTTTGT AACTGGCAAA TTAGGCTCTG GAAAGACGCT CGTTAGCGTT  
AAATAAAACA TTGACCGTTT AATCCGAGAC CTTTCTGCGA GCAATCGCAA

3901 GGTAAGATTC AGGATAAAAT TGTAGCTGGG TGCAAAATAG CAACTAATCT  
CCATTCTAAG TCCTATTTTA ACATCGACCC ACGTTTTATC GTTGATTAGA

3951 TGATTTAAGG CTTCAAAACC TCCCGCAAGT CGGGAGGTTC GCTAAAACGC  
ACTAAATTCC GAAGTTTTGG AGGGCGTTCA GCCCTCCAAG CGATTTTGCG

4001 CTCGCGTTCT TAGAATACCG GATAAGCCTT CTATTTCTGA TTTGCTTGCT  
GAGCGCAAGA ATCTTATGGC CTATTCGGAA GATAAAGACT AAACGAACGA

4051 ATTGGTCGTG GTAATGATTC CTACGACGAA AATAAAAACG GTTTGCTTGT  
TAACCAGCAC CATTACTAAG GATGCTGCTT TTATTTTTCG CAAACGAACA

4101 TCTTGATGAA TGCGGTACTT GGTTTAATAC CCGTTCATGG AATGACAAGG  
AGAACTACTT ACGCCATGAA CCAAATTATG GGCAAGTACC TTACTGTTCC

20/39

4151 AAAGACAGCC GATTATTGAT TGGTTTCTTC ATGCTCGTAA ATTGGGATGG  
 TTTCTGTCGG CTAATAACTA ACCAAAGAAG TACGAGCATT TAACCTTACC  
 4201 GATATTATTT TTCTTGTTCA GGATTTATCT ATTGTTGATA AACAGGCGCG  
 CTATAATAAA AAGAACAAGT CCTAAATAGA TAACAACATAT TTGTCCGCGC  
 4251 TTCTGCATTA GCTGAACACG TTGTTTATTG TCGCCGTCTG GACAGAATTA  
 AAGACGTAAT CGACTTGTGC AACAAATAAC AGCGGCAGAC CTGTCTTAAT  
 4301 CTTTACCCTT TGTCGGCACT TTATATTCTC TTGTTACTGG CTCAAAAATG  
 GAAATGGGAA ACAGCCGTGA AATATAAGAG AACAATGACC GAGTTTTTAC  
 4351 CCTCTGCCTA AATTACATGT TGGTGTGTGTT AAATATGGTG ATTCTCAATT  
 GGAGACGGAT TTAATGTACA ACCACAACAA TTTATACCAC TAAGAGTTAA  
 4401 AAGCCCTACT GTTGAGCGTT GGCTTTATAC TGGTAAGAAT TTATATAACG  
 TTCGGGATGA CAACTCGCAA CCGAAATATG ACCATTCTTA AATATATTGC  
 4451 CATATGACAC TAAACAGGCT TTTTCCAGTA ATTATGATTC AGGTGTTTAT  
 GTATACTGTG ATTTGTCCGA AAAAGGTCAT TAATACTAAG TCCACAAATA  
 4501 TCATATTTAA CCCCTTATTT ATCACACGGT CGGTATTTCA AACCATTAAA  
 AGTATAAATT GGGGAATAAA TAGTGTGCCA GCCATAAAGT TTGGTAATTT  
 4551 TTTAGGTCAG AAGATGAAAT TAACTAAAAT ATATTTGAAA AAGTTTTCTC  
 AAATCCAGTC TTCTACTTTA ATTGATTTTA TATAAACTTT TTCAAAAGAG  
 4601 GCGTTCTTTG TCTTGCGATA GGATTTGCAT CAGCATTTAC ATATAGTTAT  
 CGCAAGAAAC AGAACGCTAT CCTAAACGTA GTCGTAAATG TATATCAATA  
 4651 ATAACCCAAC CTAAGCCGGA GGTTAAAAAG GTAGTCTCTC AGACCTATGA  
 TATTGGGTTG GATTCGGCCT CCAATTTTTTC CATCAGAGAG TCTGGATACT  
 4701 TTTTGATAAA TTTACTATTG ACTCTTCTCA GCGTCTTAAT CTAAGCTATC  
 AAAACTATTT AAGTGATAAC TGAGAAGAGT CGCAGAATTA GATTCGATAG  
 4751 GCTATGTTTT CAAGGATTCT AAGGGAAAAT TAATTAATAG CGACGATTTA  
 CGATACAAAA GTTCCTAAGA TTCCCTTTTA ATTAATTATC GCTGCTAAAT  
 4801 CAGAAGCAAG GTTATTCCAT CACATATATT GATTTATGTA CTGTTTCAAT  
 GTCTTCGTTC CAATAAGGTA GTGTATATAA CTAAATACAT GACAAAGTTA  
 4851 TAAAAAAGGT AATTCAAATG AAATTGTTAA ATGTAATTAA TTTTGTTTTC  
 ATTTTTTCCA TTAAGTTTAC TTAAACAATT TACATTAATT AAAACAAAAG  
 4901 TTGATGTTTG TTTCATCATC TTCTTTTGCT CAAGTAATTG AAATGAATAA  
 AACTACAAAC AAAGTAGTAG AAGAAAACGA GTTCATTAAC TTTACTTATT  
 4951 TTCGCCTCTG CGCGATTTCTG TGACTTGGTA TTCAAAGCAA ACAGGTGAAT  
 AAGCGGAGAC GCGCTAAAGC ACTGAACCAT AAGTTTCGTT TGTCCACTTA

21/39

|      |            |            |            |            |             |
|------|------------|------------|------------|------------|-------------|
| 5001 | CTGTTATTGT | CTCACCTGAT | GTTAAAGGTA | CAGTGACTGT | ATATTCCTCT  |
|      | GACAATAACA | GAGTGGACTA | CAATTTCCAT | GTCACTGACA | TATAAGGAGA  |
| 5051 | GACGTTAAGC | CTGAAAATTT | ACGCAATTTT | TTTATCTCTG | TTTTACGTGC  |
|      | CTGCAATTCG | GACTTTTAAA | TGCGTTAAAG | AAATAGAGAC | AAAATGCACG  |
| 5101 | TAATAATTTT | GATATGGTTG | GCTCAATTCC | TTCCATAATT | CAGAAATATA  |
|      | ATTATTAAAA | CTATACCAAC | CGAGTTAAGG | AAGGTATTAA | GTCTTTATAT  |
| 5151 | ACCCAAATAG | TCAGGATTAT | ATTGATGAAT | TGCCATCATC | TGATATTCAG  |
|      | TGGGTTTATC | AGTCCTAATA | TAACACTTAA | ACGGTAGTAG | ACTATAAGTC  |
| 5201 | GAATATGATG | ATAATTCCGC | TCCTTCTGGT | GGTTTCTTTG | TTCCGCAAAA  |
|      | CTTATACTAC | TATTAAGGCG | AGGAAGACCA | CCAAAGAAAC | AAGGCGTTTT  |
| 5251 | TGATAATGTT | ACTCAAACAT | TTAAAATTAA | TAACGTTCGC | GCAAAGGATT  |
|      | ACTATTACAA | TGAGTTTGTA | AATTTTAATT | ATTGCAAGCG | CGTTTCCTAA  |
| 5301 | TAATAAGGGT | TGTAGAATTG | TTTGTTAAAT | CTAATACATC | TAAATCCTCA  |
|      | ATTATTCCCA | ACATCTTAAC | AAACAATTTA | GATTATGTAG | ATTTAGGAGT  |
| 5351 | AATGTATTAT | CTGTTGATGG | TTCTAACTTA | TTAGTAGTTA | GCGCCCCTAA  |
|      | TTACATAATA | GACAACTACC | AAGATTGAAT | AATCATCAAT | CGCGGGGATT  |
| 5401 | AGATATTTTA | GATAACCTTC | CGCAATTTCT | TTCTACTGTT | GATTTGCCAA  |
|      | TCTATAAAAT | CTATTGGAAG | GCGTTAAAGA | AAGATGACAA | CTAAACGGTT  |
| 5451 | CTGACCAGAT | ATTGATTGAA | GGATTAATTT | TCGAGGTTCA | GCAAGGTGAT  |
|      | GACTGGTCTA | TAACCTAATT | CCTAATTAAA | AGCTCCAAGT | CGTTCCACTA  |
| 5501 | GCTTTAGATT | TTTCCTTTGC | TGCTGGCTCT | CAGCGCGGCA | CTGTTGCTGG  |
|      | CGAAATCTAA | AAAGGAAACG | ACGACCGAGA | GTCGCGCCGT | GACAACGACC  |
| 5551 | TGGTGTTAAT | ACTGACCGTC | TAACCTCTGT | TTTATCTTCT | GCGGGTGGTT  |
|      | ACCACAATTA | TGACTGGCAG | ATTGGAGACA | AAATAGAAGA | CGCCCACCAA  |
| 5601 | CGTTCGGTAT | TTTTAACGGC | GATGTTTTAG | GGCTATCAGT | TCGCGCATTAA |
|      | GCAAGCCATA | AAAATTGCCG | CTACAAAATC | CCGATAGTCA | AGCGCGTAAT  |
| 5651 | AAGACTAATA | GCCATTCAAA | AATATTGTCT | GTGCCTCGTA | TTCTTACGCT  |
|      | TTCTGATTAT | CGGTAAGTTT | TTATAACAGA | CACGGAGCAT | AAGAATGCGA  |
| 5701 | TTCAGGTCAG | AAGGGTTCTA | TTTCTGTTGG | CCAGAATGTC | CCTTTTATTA  |
|      | AAGTCCAGTC | TTCCCAAGAT | AAAGACAACC | GGTCTTACAG | GGAATAAAT   |
| 5751 | CTGGTCGTGT | AACTGGTGAA | TCTGCCAATG | TAAATAATCC | ATTTACAGACG |
|      | GACCAGCACA | TTGACCACTT | AGACGGTTAC | ATTTATTAGG | TAAAGTCTGC  |
| 5801 | GTTGAGCGTC | AAAATGTTGG | TATTTCTATG | AGTGTTTTTC | CCGTTGCAAT  |
|      | CAACTCGCAG | TTTTACAACC | ATAAAGATAC | TCACAAAAAG | GGCAACGTTA  |

22/39

5851 GGCTGGCGGT AATATTGTTT TAGATATAAC CAGTAAGGCC GATAGTTTGA  
 CCGACCGCCA TTATAACAAA ATCTATATTG GTCATTCCGG CTATCAAAC

5901 GTTCTTCTAC TCAGGCAAGT GATGTTATTA CTAATCAAAG AAGTATTGCG  
 CAAGAAGATG AGTCCGTTCA CTACAATAAT GATTAGTTTC TTCATAACGC

5951 ACAACGGTTA ATTTGCGTGA TGGTCAGACT CTTTTGCTCG GTGGCCTCAC  
 TGTTGCCAAT TAAACGCACT ACCAGTCTGA GAAAACGAGC CACCGGAGTG

6001 TGATTACAAA AACACTTCTC AAGATTCTGG TGTGCCGTTT CTGTCTAAAA  
 ACTAATGTTT TTGTGAAGAG TTCTAAGACC ACACGGCAAG GACAGATTTT

6051 TCCCTTTAAT CGGCCTCCTG TTTAGCTCCC GTTCTGATTC TAACGAGGAA  
 AGGGAAATTA GCCGGAGGAC AAATCGAGGG CAAGACTAAG ATTGCTCCTT

6101 AGCACGTTGT ACGTGCTCGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG  
 TCGTGCAACA TGCACGAGCA GTTTCGTTGG TATCATGCGC GGGACATCGC

6151 GCGCATTAAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA  
 CGCGTAATTC GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT

6201 CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT  
 GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA

BamHI

-----

6251 CGCCACGTTT TCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGATCCCTT  
 GCGGTGCAAG AGGCCGAAAG GGGCAGTTTC AGATTTAGCC CCCTAGGGAA

6301 TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCTCCA AAAACTTGAT  
 ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGAGGT TTTTGAAC

6351 TTGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG  
 AACCCACTAC CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC

6401 CCCTTTGACG TTGGAGTCCA CGTTCCTTAA TAGTGGAATC TTGTTCCAAA  
 GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT

6451 CTGGAACAAC ACTCACAAC AACTCGGCCT ATTCTTTTGA TTTATAAGGA  
 GACCTTGTTG TGAGTGTTGA TTGAGCCGGA TAAGAAAACT AAATATTCCT

6501 TTTTGTGTCAT TTTCTGCTTA CTGGTTAAAA AATAAGCTGA TTTAACAAAT  
 AAAAACAGTA AAAGACGAAT GACCAATTTT TTATTCGACT AAATTGTTTA

6551 ATTTAACGCG AAATTTAACA AAACATTAAC GTTTACAATT TAAATATTTG  
 TAAATTGCGC TTAAATTGT TTTGTAATTG CAAATGTTAA ATTTATAAAC

6601 CTTATACAAT CATCCTGTTT TTGGGGCTTT TCTGATTATC AACCGGGGTA  
 GAATATGTTA GTAGGACAAA AACCCGAAA AGACTAATAG TTGGCCCCAT

23/39

Clai

~~~~~

6651 CATATGATTG ACATGCTAGT TTTACGATTA CCGTTCATCG ATTCTCTTGT
GTATACTAAC TGTACGATCA AAATGCTAAT GGCAAGTAGC TAAGAGAACA

6701 TTGCTCCAGA CTTTCAGGTA ATGACCTGAT AGCCTTTGTA GACCTCTCAA
AACGAGGTCT GAAAGTCCAT TACTGGACTA TCGGAAACAT CTGGAGAGTT

6751 AAATAGCTAC CCTCTCCGGC ATGAATTTAT CAGCTAGAAC GGTGAATAT
TTTATCGATG GGAGAGGCCG TACTTAAATA GTCGATCTTG CCAACTTATA

6801 CATATTGACG GTGATTTGAC TGTCTCCGGC CTTTCTCACC CGTTTGAATC
GTATAACTGC CACTAAACTG ACAGAGGCCG GAAAGAGTGG GCAAACCTAG

6851 TTTGCCTACT CATTACTCCG GCATTGCATT TAAAATATAT GAGGGTTCTA
AAACGGATGA GTAATGAGGC CGTAACGTAA ATTTTATATA CTCCCAAGAT

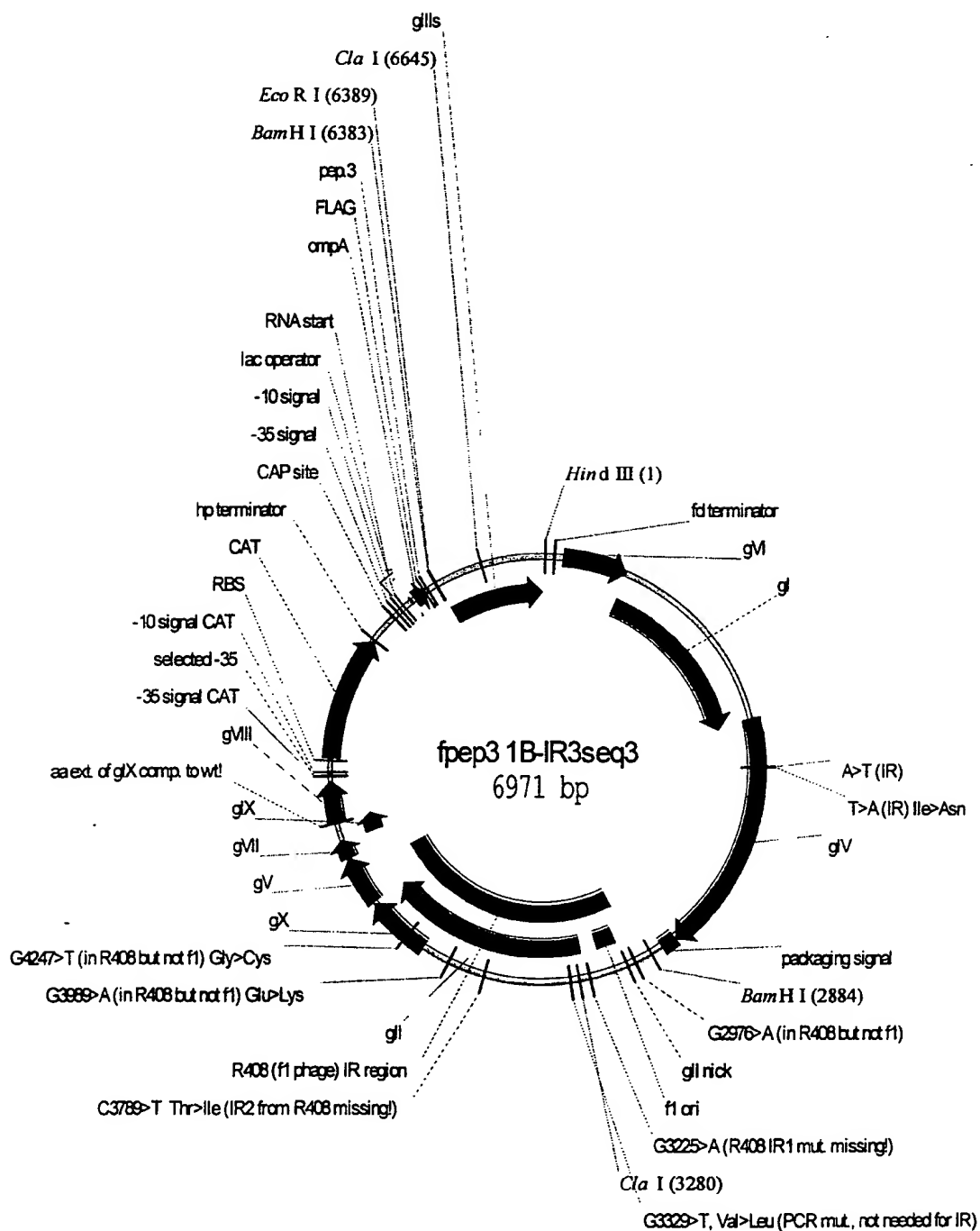
6901 AAAATTTTTA TCCCTGCGTT GAAATTAAGG CTTACCAGC AAAAGTATTA
TTTTAAAAAT AGGGACGCAA CTTTAATTCC GAAGTGGTCG TTTTCATAAT

6951 CAGGGTCATA ATGTTTTTGG TACAACCGAT TTAGCTTTAT GCTCTGAGGC
GTCCCAGTAT TACAAAACC ATGTTGGCTA AATCGAAATA CGAGACTCCG

7001 TTTATTGCTT AATTTTGCTA ACTCTCTGCC TTGCTTGAC GATTTATTGG
AAATAACGAA TTAAAACGAT TGAGAGACGG AACGAACATG CTAAATAACC

7051 ATGTT
TACAA

Figure 4



25/39

HindIII

~~~~~

```

1  AGCTTCGAGA AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA
   TCGAAGCTCT TTAAGTGGAG CTTTCGTTTC ACTATTTGGC TATGTTAATT

51  AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG AATTAATTCA ATCATGCCAG
   TCCGAGGAAA ACCTCGGAAA AAAAAACCTC TTAATTAAGT TAGTACGGTC

101 TTCTTTTGGG TATTCCTGTA TTATTGCGTT TCCTCGGTTT CCTTCTGGTA
   AAGAAAACCC ATAAGGCAAT AATAACGCAA AGGAGCCAAA GGAAGACCAT

151 ACTTTGTTTC GCTATCTGCT TACTTTCCTT AAAAAGGGCT TCGGTAAGAT
   TGAAACAAGC CGATAGACGA ATGAAAGGAA TTTTCCCGA AGCCATTCTA

201 AGCTATTGCT ATTTCAATTGT TTCTTGCTCT TATTATTGGG CTTAACTCAA
   TCGATAACGA TAAAGTAACA AAGAACGAGA ATAATAACCC GAATTGAGTT

251 TTCTTGTTGG TTATCTCTCT GATATTAGCG CACAATTACC CTCTGATTTT
   AAGAACACCC AATAGAGAGA CTATAATCGC GTGTTAATGG GAGACTAAAA

301 GTTCAGGGCG TTCAGTTAAT TCTCCCGTCT AATGCGCTTC CCTGTTTTTA
   CAAGTCCCGC AAGTCAATTA AGAGGGCAGA TTACGCGAAG GGACAAAAAT

351 TGTTATTCTC TCTGTAAAGG CTGCTATTTT CATTTTTGAC GTTAAACAAA
   ACAATAAGAG AGACATTTCC GACGATAAAA GTAAAACTG CAATTTGTTT

401 AAATCGTTTC TTATTTGGAT TGGGATAAAT AAATATGGCT GTTTATTTTG
   TTTAGCAAAG AATAAACCTA ACCCTATTTA TTTATACCGA CAAATAAAAC

451 TAACTGGCAA ATTAGGCTCT GGAAAGACGC TCGTTAGCGT TGGTAAGATT
   ATTGACCGTT TAATCCGAGA CTTTCTGCG AGCAATCGCA ACCATTCTAA

501 CAGGATAAAA TTGTAGCTGG GTGCAAAATA GCAACTAATC TTGATTTAAG
   GTCCTATTTT AACATCGACC CACGTTTTAT CGTTGATTAG AACTAAATTC

551 GCTTCAAAAC CTCCCGCAAG TCGGGAGGTT CGCTAAAACG CCTCGCGTTC
   CGAAGTTTTG GAGGGCGTTC AGCCCTCCAA GCGATTTTGC GGAGCGCAAG

601 TTAGAATACC GGATAAGCCT TCTATTTCTG ATTTGCTTGC TATTGGTCGT
   AATCTTATGG CCTATTCGGA AGATAAAGAC TAAACGAACG ATAACCAGCA

651 GGTAATGATT CCTACGACGA AAATAAAAC GGTTCGCTTG TTCTTGATGA
   CCATTACTAA GGATGCTGCT TTTATTTTTG CCAAACGAAC AAGAACTACT

701 ATGCGGTACT TGGTTTAATA CCCGTTTCATG GAATGACAAG GAAAGACAGC
   TACGCCATGA ACCAAATTAT GGGCAAGTAC CTTACTGTTC CTTTCTGTTCG

751 CGATTATTGA TTGGTTTCTT CATGCTCGTA AATTGGGATG GGATATTATT
   GCTAATAACT AACCAAAGAA GTACGAGCAT TTAACCCTAC CCTATAATAA

```

26/39

|      |            |            |            |            |            |
|------|------------|------------|------------|------------|------------|
| 801  | TTTCTTGTTT | AGGATTTATC | TATTGTTGAT | AAACAGGCGC | GTTCTGCATT |
|      | AAAGAACAAG | TCCTAAATAG | ATAACAATA  | TTTGTCCGCG | CAAGACGTAA |
| 851  | AGCTGAACAC | GTTGTTTATT | GTCGCCGTCT | GGACAGAATT | ACTTTACCCT |
|      | TCGACTTGTG | CAACAAATA  | CAGCGGCAGA | CCTGTCTTAA | TGAAATGGGA |
| 901  | TTGTCGGCAC | TTTATATTCT | CTTGTTACTG | GCTCAAAAAT | GCCTCTGCCT |
|      | AACAGCCGTG | AAATATAAGA | GAACAATGAC | CGAGTTTTTA | CGGAGACGGA |
| 951  | AAATTACATG | TTGGTGTTGT | TAAATATGGT | GATTCTCAAT | TAAGCCCTAC |
|      | TTTAATGTAC | AACCACAACA | ATTTATACCA | CTAAGAGTTA | ATTGCGGATG |
| 1001 | TGTTGAGCGT | TGGCTTTATA | CTGGTAAGAA | TTTATATAAC | GCATATGACA |
|      | ACAACCTCGA | ACCGAAATAT | GACCATTTCT | AAATATATTG | CGTATACTGT |
| 1051 | CTAAACAGGC | TTTTTCCAGT | AATTATGATT | CAGGTGTTTA | TTCATATTTA |
|      | GATTTGTCCG | AAAAAGGTCA | TTAATACTAA | GTCCACAAAT | AAGTATAAAT |
| 1101 | ACCCCTTATT | TATCACACGG | TCGGTATTTT | AAACCATTAA | ATTTAGGTCA |
|      | TGGGGAATAA | ATAGTGTGCC | AGCCATAAAG | TTTGGTAATT | TAAATCCAGT |
| 1151 | GAAGATGAAA | TTAACTAAAA | TATATTTGAA | AAAGTTTTCT | CGCGTTCTTT |
|      | CTTCTACTTT | AATTGATTTT | ATATAAACTT | TTTCAAAAGA | GCGCAAGAAA |
| 1201 | GTCTTGCGAT | AGGATTTGCA | TCAGCATTTA | CATATAGTTA | TATAACCCAA |
|      | CAGAACGCTA | TCCTAAACGT | AGTCGTAAAT | GTATATCAAT | ATATTGGGTT |
| 1251 | CCTAAGCCGG | AGGTAAAAA  | GGTAGTCTCT | CAGACCTATG | ATTTTGATAA |
|      | GGATTGCGCC | TCCAATTTTT | CCATCAGAGA | GTCTGGATAC | TAAAACTATT |
| 1301 | ATTCATATT  | GACTCTTCTC | AGCGTCTTAA | TCTAAGCTAT | CGCTATGTTT |
|      | TAAGTGATAA | CTGAGAAGAG | TCGAGAATT  | AGATTGATA  | GCGATACAAA |
| 1351 | TCAAGGATTC | TAAGGGAAAA | TTAATTAATA | GCGACGATTT | ACAGAAGCAA |
|      | AGTTCCTAAG | ATTCCCTTTT | AATTAATTAT | CGCTGCTAAA | TGTCTTCGTT |
| 1401 | GGTTATTCCA | TCACATATAT | TGATTTATGT | ACTGTTTCAA | TTAAAAAAGG |
|      | CCAATAAGGT | AGTGTATATA | ACTAAATACA | TGACAAAGTT | AATTTTTC   |
| 1451 | TAATTCAAAT | GAAATTGTTA | AATGTAATTA | ATTTTGTTTT | CTTGATGTTT |
|      | ATTAAGTTTA | CTTTAACAAT | TTACATTAAT | TAAAACAAAA | GAACACAAA  |
| 1501 | GTTTCATCAT | CTTCTTTTGC | TCAAGTAATT | GAAATGAATA | ATTCGCCTCT |
|      | CAAAGTAGTA | GAAGAAAACG | AGTTCATTAA | CTTTACTTAT | TAAGCGGAGA |
| 1551 | GCGCGATTTT | GTGACTTGGT | ATTCAAAGCA | AACAGGTGAA | TCTGTTATTG |
|      | CGCGCTAAAG | CACTGAACCA | TAAGTTTCGT | TTGTCCACTT | AGACAATAAC |
| 1601 | TCTCACCTGA | TGTTAAAGGT | ACAGTGAAGT | TATATTCCTC | TGACGTTAAG |
|      | AGAGTGGAAG | ACAATTTCCT | TGTCAGTGAC | ATATAAGGAG | ACTGCAATTC |

27/39

|      |             |            |             |            |            |
|------|-------------|------------|-------------|------------|------------|
| 1651 | CCTGAAAATT  | TACGCAATTT | CTTTATCTCT  | GTTTTACGTG | CTAATAATTT |
|      | GGACTTTTAA  | ATGCGTTAAA | GAAATAGAGA  | CAAATGCAC  | GATTATTAAA |
| 1701 | TGATATGGTT  | GGCTCTAATC | CTTCCATAAT  | TCAGAAATAT | AACCCAAATA |
|      | ACTATACCAA  | CCGAGATTAG | GAAGGTATTA  | AGTCTTTATA | TTGGGTTTAT |
| 1751 | GTCAGGATTA  | TATTGATGAA | TTGCCATCAT  | CTGATATTCA | GGAATATGAT |
|      | CAGTCCTAAT  | ATAACTACTT | AACGGTAGTA  | GACTATAAGT | CCTTATACTA |
| 1801 | GATAATTCCG  | CTCCTTCTGG | TGGTTTCTTT  | GTTCCGCAAA | ATGATAATGT |
|      | CTATTAAGGC  | GAGGAAGACC | ACCAAAGAAA  | CAAGGCGTTT | TACTATTACA |
| 1851 | TACTCAAACA  | TTTAAAATTA | ATAACGTTTCG | CGCAAAGGAT | TTAATAAGGG |
|      | ATGAGTTTGT  | AAATTTTAAT | TATTGCAAGC  | GCGTTTCCTA | AATTATTCCC |
| 1901 | TTGTAGAATT  | GTTTGTTAAA | TCTAATACAT  | CTAAATCCTC | AAATGTATTA |
|      | AACATCTTAA  | CAAACAATTT | AGATTATGTA  | GATTTAGGAG | TTTACATAAT |
| 1951 | TCTGTTGATG  | GTTCTAACTT | ATTAGTAGTT  | AGCGCCCCTA | AAGATATTTT |
|      | AGACAACCTAC | CAAGATTGAA | TAATCATCAA  | TCGCGGGGAT | TTCTATAAAA |
| 2001 | AGATAACCTT  | CCGCAATTTT | TTTCTACTGT  | TGATTTGCCA | ACTGACCAGA |
|      | TCTATTGGAA  | GGCGTTAAAG | AAAGATGACA  | ACTAAACGGT | TGACTGGTCT |
| 2051 | TATTGATTGA  | AGGATTAATT | TTCGAGGTTC  | AGCAAGGTGA | TGCTTTAGAT |
|      | ATAACTAACT  | TCCTAATTAA | AAGCTCCAAG  | TCGTTCCACT | ACGAAATCTA |
| 2101 | TTTTCCTTTG  | CTGCTGGCTC | TCAGCGCGGC  | ACTGTTGCTG | GTGGTGTTAA |
|      | AAAAGGAAAC  | GACGACCGAG | AGTCGCGCCG  | TGACAACGAC | CACCACAATT |
| 2151 | TACTGACCGT  | CTAACCTCTG | TTTTATCTTC  | TGCGGGTGGT | TCGTTCCGTA |
|      | ATGACTGGCA  | GATTGGAGAC | AAAATAGAAG  | ACGCCACCA  | AGCAAGCCAT |
| 2201 | TTTTTAACGG  | CGATGTTTTA | GGGCTATCAG  | TCGCGCATT  | AAAGACTAAT |
|      | AAAAATTGCC  | GCTACAAAAT | CCCATAGTC   | AAGCGCGTAA | TTTCTGATTA |
| 2251 | AGCCATTCAA  | AAATATTGTC | TGTGCCTCGT  | ATTCTTACGC | TTTCAGGTCA |
|      | TCGGTAAGTT  | TTTATAACAG | ACACGGAGCA  | TAAGAATGCG | AAAGTCCAGT |
| 2301 | GAAGGGTTCT  | ATTTCTGTTG | GCCAGAATGT  | CCCTTTTATT | ACTGGTCGTG |
|      | CTTCCCAAGA  | TAAAGACAAC | CGGTCTTACA  | GGGAAAATAA | TGACCAGCAC |
| 2351 | TAACTGGTGA  | ATCTGCCAAT | GTAAATAATC  | CATTTACAGC | AATTGAGCGT |
|      | ATTGACCACT  | TAGACGGTTA | CATTTATTAG  | GTAAAGTCTG | TTAACTCGCA |
| 2401 | CAAATGTTG   | GTATTTCTAT | GAGTGTTTTT  | CCCGTTGCAA | TGGCTGGCGG |
|      | GTTTTACAAC  | CATAAAGATA | CTCACAAAAA  | GGGCAACGTT | ACCGACCGCC |
| 2451 | TAATATTGTT  | TTAGATATAA | CCAGTAAGGC  | CGATAGTTTG | AGTTCTTCTA |
|      | ATTATAACAA  | AATCTATATT | GGTCATTCCG  | GCTATCAAAC | TCAAGAAGAT |

28/39

2501 CTCAGGCAAG TGATGTTATT ACTAATCAAA GAAGTATTGC GACAACGGTT  
GAGTCCGTTT ACTACAATAA TGATTAGTTT CTTCATAACG CTGTTGCCAA

2551 AATTTGCGTG ATGGTCAGAC TCTTTTGCTC GGTGGCCTCA CTGATTACAA  
TTAAACGCAC TACCAGTCTG AGAAAACGAG CCACCGGAGT GACTAATGTT

2601 AAACACTTCT CAAGATTCTG GTGTGCCGTT CCTGTCTAAA ATCCCTTTAA  
TTTGTGAAGA GTTCTAAGAC CACACGGCAA GGACAGATTT TAGGGAAATT

2651 TCGGCCTCCT GTTTAGCTCC CGTTCTGATT CTAACGAGGA AAGCACGTTG  
AGCCGGAGGA CAAATCGAGG GCAAGACTAA GATTGCTCCT TTCGTGCAAC

2701 TACGTGCTCG TCAAAGCAAC CATAGTACGC GCCCTGTAGC GGCGCATTAA  
ATGCACGAGC AGTTTCGTTG GTATCATGCG CGGGACATCG CCGCGTAATT

2751 GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC  
CGCGCCGCC ACACCACCAA TGCGCGTCGC ACTGGCGATG TGAACGGTCG

2801 GCCCTAGCGC CCGCTCCTTT CGCTTCTTTC CCTTCCTTTC TCGCCACGTT  
CGGGATCGCG GGCGAGGAAA GCGAAAGAAG GGAAGGAAAG AGCGGTGCAA

BamHI

-----

2851 CTCCGGCTTT CCCCCTCAAG CTCTAAATCG GGGGATCCCT TTAGGGTTCC  
GAGGCCGAAA GGGGCAGTTC GAGATTTAGC CCCCTAGGGA AATCCCAAGG

2901 GATTTAGTGC TTTACGGCAC CTCGACCTCC AAAAACTTGA TTTGGGTGAT  
CTAAATCACG AAATGCCGTG GAGCTGGAGG TTTTGAAC TAAACCACTA

2951 GGTTCACGTA GTGGGCCATC GCCCTAATAG ACGGTTTTTC GCCCTTTGAC  
CCAAGTGCAT CACCCGGTAG CGGGATTATC TGCCAAAAAG CGGGAAACTG

3001 GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA ACTGGAACAA  
CAACCTCAGG TGCAAGAAAT TATCACCTGA GAACAAGGTT TGACCTTGTT

3051 CACTCAACCC TATCTCGGTC TATTCTTTTG ATTTATAAGG GATTTTGCCG  
GTGAGTTGGG ATAGAGCCAG ATAAGAAAAC TAAATATTCC CTAAAACGGC

3101 ATTTCCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC  
TAAAGCCGGA TAACCAATTT TTTACTCGAC TAAATTGTTT TTAAATTGCG

3151 GAATTTTAAAC AAAATATTAA CGTTTACAAT TTAAATATTT GCTTATACAA  
CTTAAAAATTG TTTTATAATT GCAAATGTTA AATTTATAAA CGAATATGTT

3201 TCTTCCTGTT TTTGGGGCTT TTCTGATTAT CAACCGGGGT ACATATGATT  
AGAAGGACAA AAACCCCGAA AAGACTAATA GTTGGCCCCA TGTATACTAA

ClaI

-----

3251 GACATGCTAG TTTTACGATT ACCGTTTCATC GATTCTCTTG TTTGCTCCAG  
CTGTACGATC AAAATGCTAA TGGCAAGTAG CTAAGAGAAC AAACGAGGTC

29/39

3301 ACTCTCAGGC AATGACCTGA TAGCCTTTTT AGACCTCTCA AAAATAGCTA  
TGAGAGTCCG TTACTGGACT ATCGGAAAAA TCTGGAGAGT TTTTATCGAT

3351 CCCTCTCCGG CATGAATTTA TCAGCTAGAA CGGTTGAATA TCATATTGAT  
GGGAGAGGCC GTACTTAAAT AGTCGATCTT GCCAACTTAT AGTATAACTA

3401 GGTGATTTGA CTGTCTCCGG CCTTTCTCAC CCGTTTGAAT CTTTACCTAC  
CCACTAAACT GACAGAGGCC GGAAAGAGTG GGCAAACCTTA GAAATGGATG

3451 ACATTACTCA GGCATTGCAT TTAAAATATA TGAGGGTTCT AAAAATTTTT  
TGTAATGAGT CCGTAACGTA AATTTTATAT ACTCCCAAGA TTTTAAAAA

3501 ATCCTTGCGT TGAAATAAAG GCTTCTCCCG CAAAAGTATT ACAGGGTCAT  
TAGGAACGCA ACTTTATTTT CGAAGAGGGC GTTTTCATAA TGTCCCAGTA

3551 AATGTTTTTG GTACAACCGA TTTAGCTTTA TGCTCTGAGG CTTTATTGCT  
TTACAAAAAC CATGTTGGCT AAATCGAAAT ACGAGACTCC GAAATAACGA

3601 TAATTTTGCT AATTCCTTGC CTTGCCTGTA TGATTTATTG GATGTTAACG  
ATTAAACGA TTAAGAAACG GAACGGACAT ACTAAATAAC CTACAATTGC

3651 CTACTACTAT TAGTAGAATT GATGCCACCT TTTCAGCTCG CGCCCCAAAT  
GATGATGATA ATCATCTTAA CTACGGTGGA AAAGTCGAGC GCGGGGTTTA

3701 GAAAATATAG CTAAACAGGT TATTGACCAT TTGCGAAATG TATCTAATGG  
CTTTTATATC GATTTGTCCA ATAAGTGGTA AACGCTTTAC ATAGATTACC

3751 TCAAACATAA TCTACTCGTT CGCAGAATTG GGAATCAACT GTTACATGGA  
AGTTTGATTT AGATGAGCAA GCGTCTTAAC CCTTAGTTGA CAATGTACCT

3801 ATGAAACTTC CAGACACCGT ACTTTAGTTG CATATTTAAA ACATGTTGAG  
TACTTTGAAG GTCTGTGGCA TGAAATCAAC GTATAAATTT TGTACAACCTC

3851 CTACAGCACC AGATCCAGCA ATTAAGCTCT AAGCCATCCG CAAAAATGAC  
GATGTCGTGG TCTAGGTCGT TAATTCGAGA TTCGGTAGGC GTTTTTACTG

3901 CTCTTATCAA AAGGAGCAAT TAAAGGTACT CTCTAATCCT GACCTGTTGG  
GAGAATAGTT TTCCTCGTTA ATTTCCATGA GAGATTAGGA CTGGACAACC

3951 AGTTTGCTTC CGGTCTGGTT CGCTTTGAAG CTCGAATTAA AACGCGATAT  
TCAAACGAAG GCCAGACCAA GCGAAACTTC GAGCTTAATT TTGCGCTATA

4001 TTGAAGTCTT TCGGGCTTCC TCTTAATCTT TTTGATGCAA TCCGCTTTGC  
AACTTCAGAA AGCCCGAAGG AGAATTAGAA AAACTACGTT AGGCGAAACG

4051 TTCTGACTAT AATAGTCAGG GTAAAGACCT GATTTTTGAT TTATGGTCAT  
AAGACTGATA TTATCAGTCC CATTTCTGGA CTAAAACTA AATACCAGTA

4101 TCTCGTTTTT TGAAGTGTTC AAAGCATTTG AGGGGGATTC AATGAATATT  
AGAGCAAAAG ACTTGACAAA TTTCGTAAAC TCCCCCTAAG TTACTTATAA

30/39

4151 TATGACGATT CCGCAGTATT GGACGCTATC CAGTCTAAAC ATTTTACTAT  
 ATACTGCTAA GGCCTCATAA CCTGCGATAG GTCAGATTTG TAAAATGATA  
 4201 TACCCCTCTT GGCAAACTT CTTTTCGAAA AGCCTCTCGC TATTTTGTG  
 ATGGGGGAGA CCGTTTGTAA GAAAACGTTT TCGGAGAGCG ATAAAAACAA  
 4251 TTTATCGTCG TCTGGTAAAC GAGGGTTATG ATAGTGTTCG TCTTACTATG  
 AAATAGCAGC AGACCATTG CTCCCAATAC TATCACAACG AGAATGATAC  
 4301 CCTCGTAATT CCTTTTGGCG TTATGTATCT GCATTAGTTG AATGTGGTAT  
 GGAGCATTAA GGAAAACCGC AATACATAGA CGTAATCAAC TTACACCATA  
 4351 TCCTAAATCT CAACTGATGA ATCTTCTAC CTGTAATAAT GTTGTTCGGT  
 AGGATTTAGA GTTGACTACT TAGAAAAGATG GACATTATTA CAACAAGGCA  
 4401 TAGTTCGTTT TATTAACGTA GATTTTCTT CCCAACGTCC TGACTGGTAT  
 ATCAAGCAAA ATAATTGCAT CTAAAAAGAA GGGTTGCAGG ACTGACCATA  
 4451 AATGAGCCAG TTCTTAAAT CGCATAAGGT AATCACAAT GATTAAAGTT  
 TTAATCGGTC AAGAATTTTA GCGTATTCCA TTAAGTGTTA CTAATTTCAA  
 4501 GAAATTAAAC CATCTCAAGC GCAATTCAT ACCCGTTCTG GTGTTTCTCG  
 CTTTAATTTG GTAGAGTTCG CGTTAAGTGA TGGGCAAGAC CACAAAGAGC  
 4551 TCAGGGCAAG CCTTATTCAC TGAATGAGCA GCTTTGTTAC GTTGATTTGG  
 AGTCCCGTTC GGAATAAGTG ACTTACTCGT CGAAACAATG CAACTAAACC  
 4601 GTAATGAATA TCCGGTGCTT GTCAAGATTA CTCTTGATGA AGGTCAGCCA  
 CATTACTTAT AGGCCACGAA CAGTTCTAAT GAGAACTACT TCCAGTCGGT  
 4651 GCCTATGCGC CTGGTCTGTA CACCGTGCACT CTGTCCTCGT TCAAAGTTGG  
 CGGATACGCG GACCAGACAT GTGGCACGTA GACAGGAGCA AGTTTCAACC  
 4701 TCAGTTCGGT TCTCTTATGA TTGACCGTCT GCGCCTCGTT CCGGCTAAGT  
 AGTCAAGCCA AGAGAATACT AACTGGCAGA CGCGGAGCAA GGCCGATTCA  
 4751 AACATGGAGC AGGTCGCGGA TTTCGACACA ATTTATCAGG CGATGATACA  
 TTGTACCTCG TCCAGCGCCT AAAGCTGTGT TAAATAGTCC GCTACTATGT  
 4801 AATCTCCGTT GTACTTTGTT TCGCGCTTGG TATAATCGCT GGGGGTCAAA  
 TTAGAGGCAA CATGAAACAA AGCGCGAACC ATATTAGCGA CCCCCAGTTT  
 4851 GATGAGTGTT TTAGTGTTAT CTTTCGCCTC TTTCGTTTTA GGTTGGTGCC  
 CTAATCACA AATCACATAA GAAAGCGGAG AAAGCAAAAT CCAACCACGG  
 4901 TTCGTAGTGG CATTACGTAT TTTACCCGTT TAATGGAAAC TTCCTCATGC  
 AAGCATCACC GTAATGCATA AAATGGGCAA ATTACCTTTG AAGGAGTACG  
 4951 GTAAGTCTTT AGTCCTCAAA GCCTCCGTAG CCGTTGCTAC CCTCGTTCCG  
 CATTCAAGAA TCAGGAGTTT CGGAGGCATC GGCAACGATG GGAGCAAGGC

31/39

|      |             |            |             |            |             |
|------|-------------|------------|-------------|------------|-------------|
| 5001 | ATGCTGTCTT  | TCGCTGCTGA | GGGTGACGAT  | CCCGCAAAAG | CGGCCTTTGA  |
|      | TACGACAGAA  | AGCGACGACT | CCCCTGCTA   | GGGCGTTTTT | GCCGGAAACT  |
| 5051 | CTCCCTGCAA  | GCCTCAGCGA | CCGAATATAT  | CGGTTATGCG | TGGGCGATGG  |
|      | GAGGGACGTT  | CGGAGTCGCT | GGCTTATATA  | GCCAATACGC | ACCCGCTACC  |
| 5101 | TTGTTGTCAT  | TGTCGGCGCA | ACTATCGGTA  | TCAAGCTGTT | TAAGAAATTC  |
|      | AACAACAGTA  | ACAGCCGCGT | TGATAGCCAT  | AGTTCGACAA | ATTCTTTAAG  |
| 5151 | ACCTCGAAAG  | CAAGCTGATA | AAGGAGGTTT  | CTCGATCGAG | ACGTTGGGTG  |
|      | TGGAGCTTTC  | GTCGACTAT  | TTCTTCCAAA  | GAGCTAGCTC | TGCAACCCAC  |
| 5201 | AGGTTCCAAC  | TTTCACCATA | ATGAAATAAG  | ATCACTACCG | GGCGTATTTT  |
|      | TCCAAGGTTG  | AAAGTGGTAT | TACTTTATTTC | TAGTGATGGC | CCGCATAAAA  |
| 5251 | TTGAGTTATC  | GAGATTTTCA | GGAGCTAAGG  | AAGCTAAAT  | GGAGAAAAAA  |
|      | AACTCAATAG  | CTCTAAAAGT | CCTCGATTCC  | TTCGATTTTA | CCTCTTTTTT  |
| 5301 | ATCACTGGAT  | ATACCACCGT | TGATATATCC  | CAATGGCATC | GTAAAGAACA  |
|      | TAGTGACCTA  | TATGGTGGCA | ACTATATAGG  | GTTACCGTAG | CATTTCTTGT  |
| 5351 | TTTTGAGGCA  | TTTCAGTCAG | TTGCTCAATG  | TACCTATAAC | CAGACCGTTC  |
|      | AAAACCTCCGT | AAAGTCAGTC | AACGAGTTAC  | ATGGATATTG | GTCTGGCAAG  |
| 5401 | AGCTGGATAT  | TACGGCCTTT | TTAAAGACCG  | TAAAGAAAAA | TAAGCACAAG  |
|      | TCGACCTATA  | ATGCCGAAA  | AATTTCTGGC  | ATTTCTTTTT | ATTCTGTGTC  |
| 5451 | TTTTATCCGG  | CCTTTATTCA | CATTCTTGCC  | CGCCTGATGA | ATGCTCATCC  |
|      | AAAATAGGCC  | GGAAATAAGT | GTAAGAACGG  | GCGGACTACT | TACGAGTAGG  |
| 5501 | GGAGTTCCGT  | ATGGCAATGA | AAGACGGTGA  | GCTGGTGATA | TGGGATAGTG  |
|      | CCTCAAGGCA  | TACCGTTACT | TTCTGCCACT  | CGACCACTAT | ACCCTATCAC  |
| 5551 | TTCACCCTTG  | TTACACCGTT | TTCCATGAGC  | AAACTGAAAC | GTTTTTCATCG |
|      | AAGTGGGAAC  | AATGTGGCAA | AAGGTACTCG  | TTTGACTTTG | CAAAAGTAGC  |
| 5601 | CTCTGGAGTG  | AATACCACGA | CGATTTCCGG  | CAGTTTCTAC | ACATATATTC  |
|      | GAGACCTCAC  | TTATGGTGCT | GCTAAAGGCC  | GTCAAAGATG | TGTATATAAG  |
| 5651 | GCAAGATGTG  | GCGTGTTACG | GTGAAAACCT  | GGCCTATTTT | CCTAAAGGGT  |
|      | CGTTCTACAC  | CGCACAATGC | CACTTTTTGA  | CCGATAAAG  | GGATTTCCCA  |
| 5701 | TTATTGAGAA  | TATGTTTTTC | GTCTCAGCCA  | ATCCCTGGGT | GAGTTTCACC  |
|      | AATAACTCTT  | ATACAAAAAG | CAGAGTCGGT  | TAGGGACCCA | CTCAAAGTGG  |
| 5751 | AGTTTTGATT  | TAAACGTAGC | CAATATGGAC  | AACTTCTTCG | CCCCCGTTTT  |
|      | TCAAACTAA   | ATTTGCATCG | GTTATACCTG  | TTGAAGAAGC | GGGGGCAAAA  |
| 5801 | CACTATGGGC  | AAATATTATA | CGCAAGGCGA  | CAAGGTGCTG | ATGCCGCTGG  |
|      | GTGATACCCG  | TTTATAATAT | GCGTTCCGCT  | GTTCCACGAC | TACGGCGACC  |



32/39

|      |            |             |            |             |            |
|------|------------|-------------|------------|-------------|------------|
| 5851 | CGATTCAGGT | TCATCATGCC  | GTTTGTGATG | GCTTCCATGT  | CGGCAGAATG |
|      | GCTAAGTCCA | AGTAGTACGG  | CAAACACTAC | CGAAGGTACA  | GCCGTCTTAC |
| 5901 | CTTAATGAAT | TACAACAGTA  | CTGCGATGAG | TGGCAGGGCG  | GGGCGTAATT |
|      | GAATTACTTA | ATGTTGTCAT  | GACGCTACTC | ACCGTCCCCG  | CCCCGATTAA |
| 5951 | TTTTTAAGGC | AGTTATTGGT  | GCCCTTAAAC | GCCTGGTGCT  | AGCCTGAGGC |
|      | AAAAATTCCG | TCAATAACCA  | CGGGAATTTG | CGGACCACGA  | TCGGACTCCG |
| 6001 | CAGTTTGCTC | AGGCTCTCCC  | CGTGGAGGTA | ATAATTGCTC  | GACCGATAAA |
|      | GTCAAACGAG | TCCGAGAGGG  | GCACCTCCAT | TATTAACGAG  | CTGGCTATTT |
| 6051 | AGCGGCTTCC | TGACAGGAGG  | CCGTTTTGTT | TTGCAGCCCA  | CCTCAACGCA |
|      | TCGCCGAAGG | ACTGTCCTCC  | GGCAAAACAA | AACGTCGGGT  | GGAGTTGCGT |
| 6101 | ATTAATGTGA | GTTAGCTCAC  | TCATTAGGCA | CCCCAGGCTT  | TACACTTTAT |
|      | TAATTACACT | CAATCGAGTG  | AGTAATCCGT | GGGGTCCGAA  | ATGTGAAATA |
| 6151 | GCTTCCGGCT | CGTATGTTGT  | GTGGAATTGT | GAGCGGATAA  | CAATTTCACA |
|      | CGAAGGCCGA | GCATACAACA  | CACCTTAACA | CTCGCCTATT  | GTTAAAGTGT |
| 6201 | CAGGAAACAG | CTATGACCAT  | GATTACGAAT | TTCTAGATAA  | CGAGGGCAAA |
|      | GTCCTTTGTC | GATACTGGTA  | CTAATGCTTA | AAGATCTATT  | GCTCCCGTTT |
| 6251 | AAATGAAAAA | GACAGCTATC  | GCGATTGCAG | TGGCACTGGC  | TGGTTTCGCT |
|      | TTTACTTTTT | CTGTCGATAG  | CGCTAACGTC | ACCGTGACCG  | ACCAAAGCGA |
| 6301 | ACCGTAGCGC | AGGCCGACTA  | CAAAGATGTC | GACTGTATTG  | TTTATCATGC |
|      | TGGCATCGCG | TCCGGCTGAT  | GTTTCTACAG | CTGACATAAC  | AAATAGTACG |
|      |            |             |            | BamHI EcoRI |            |
|      |            |             |            | ~~~~~       |            |
| 6351 | TCATTATCTT | GTTGCTAAGT  | GTGGTGGTGG | AGGATCCGAA  | TTCAATGCTG |
|      | AGTAATAGAA | CAACGATTCA  | CACCACCACC | TCCTAGGCTT  | AAGTTACGAC |
| 6401 | GCGGCGGCTC | TGGTGGTGGT  | TCTGGTGGCG | GCTCTGAGGG  | TGGTGGCTCT |
|      | CGCCGCCGAG | ACCACCACCA  | AGACCACCGC | CGAGACTCCC  | ACCACCAGAG |
| 6451 | GAGGGTGGCG | GTTCTGAGGG  | TGGCGGCTCT | GAGGGAGGCG  | GTTCCGGTGG |
|      | CTCCCACCGC | CAAGACTCCC  | ACCGCCGAGA | CTCCCTCCGC  | CAAGGCCACC |
| 6501 | TGGCTCTGGT | TCCGGTGATT  | TTGATTATGA | AAAGATGGCA  | AACGCTAATA |
|      | ACCGAGACCA | AGGCCACTAA  | AACTAATACT | TTTCTACCGT  | TTGCGATTAT |
| 6551 | AGGGGGCTAT | GACCGAAAAAT | GCCGATGAAA | ACGCGCTACA  | GTCTGACGCT |
|      | TCCCCCGATA | CTGGCTTTTA  | CGGCTACTTT | TGCGCGATGT  | CAGACTGCGA |

33/39

ClaI

-----

6601 AAAGGCAAAC TTGATTCTGT CGCTACTGAT TACGGTGCTG CTATCGATGG  
TTTCCGTTTG AACTAAGACA GCGATGACTA ATGCCACGAC GATAGCTACC

6651 TTTCAATTGGT GACGTTTCCG GCCTTGCTAA TGGTAATGGT GCTACTGGTG  
AAAGTAACCA CTGCAAAGGC CGGAACGATT ACCATTACCA CGATGACCAC

6701 ATTTTGCTGG CTCTAATTCC CAAATGGCTC AAGTCGGTGA CGGTGATAAT  
TAAAACGACC GAGATTAAGG GTTTACCGAG TTCAGCCACT GCCACTATTA

6751 TCACCTTTAA TGAATAATTT CCGTCAATAT TTACCTTCCC TCCCTCAATC  
AGTGGAATTT ACTTATTAAA GGCAGTTATA AATGGAAGGG AGGGAGTTAG

6801 GGTGGAATGT CGCCCTTTTG TCTTTGGCGC TGGTAAACCA TATGAATTTT  
CCAACCTTACA GCGGGAAGAC AGAAACCGCG ACCATTTGGT ATACTTAAAA

6851 CTATTGATTG TGACAAAATA AACTTATTCC GTGGTGTCTT TCGGTTTCTT  
GATAACTAAC ACTGTTTTAT TTGAATAAGG CACCACAGAA ACGCAAAGAA

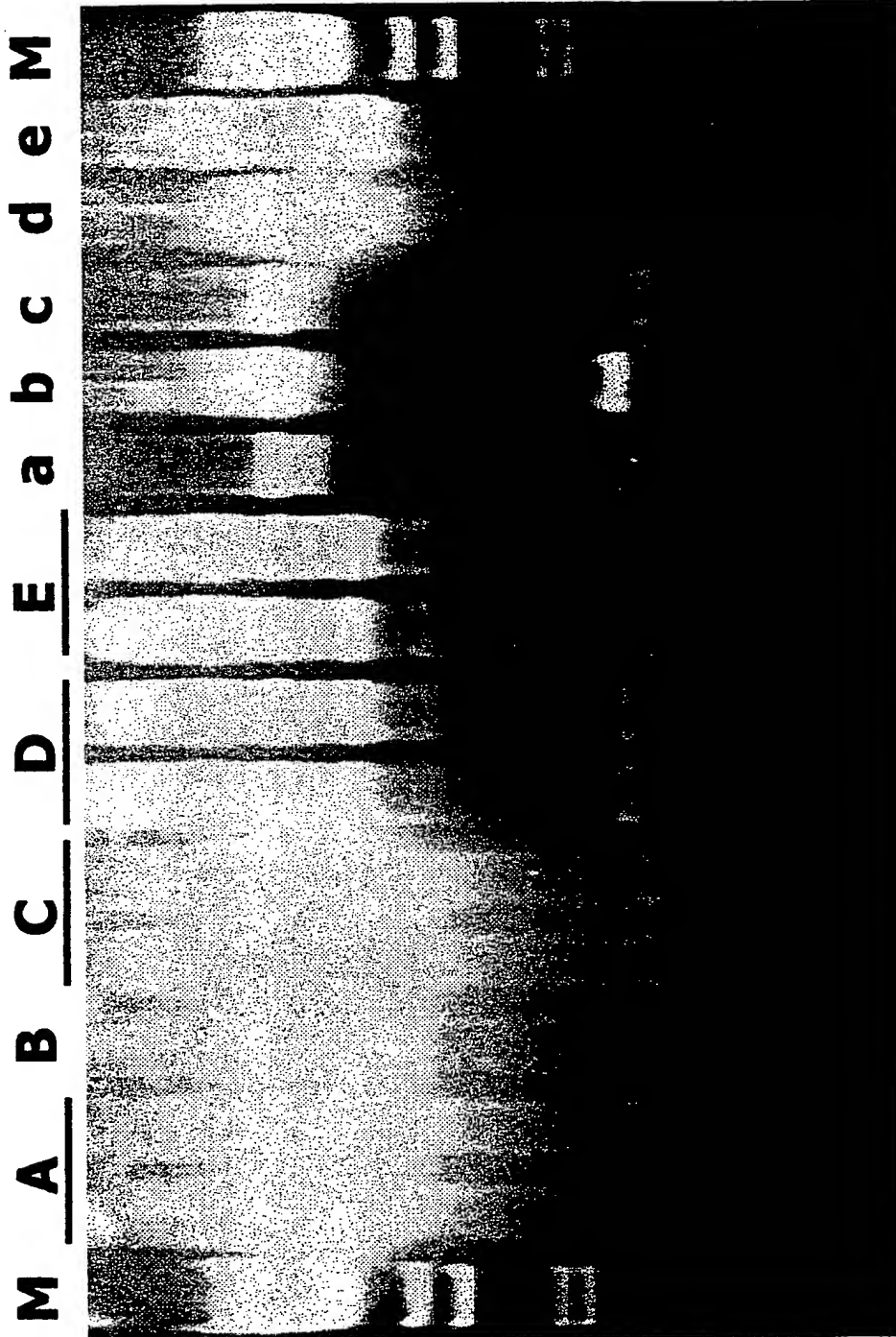
6901 TTATATGTTG CCACCTTTAT GTATGTATTT TCTACGTTTG CTAACATACT  
AATATACAAC GGTGGAATA CATACTAAA AGATGCAAAC GATTGTATGA

HindIII

6951 GCGTAATAAG GAGTCTTGAT A  
CGCATTATTC CTCAGAACTA T

34/39

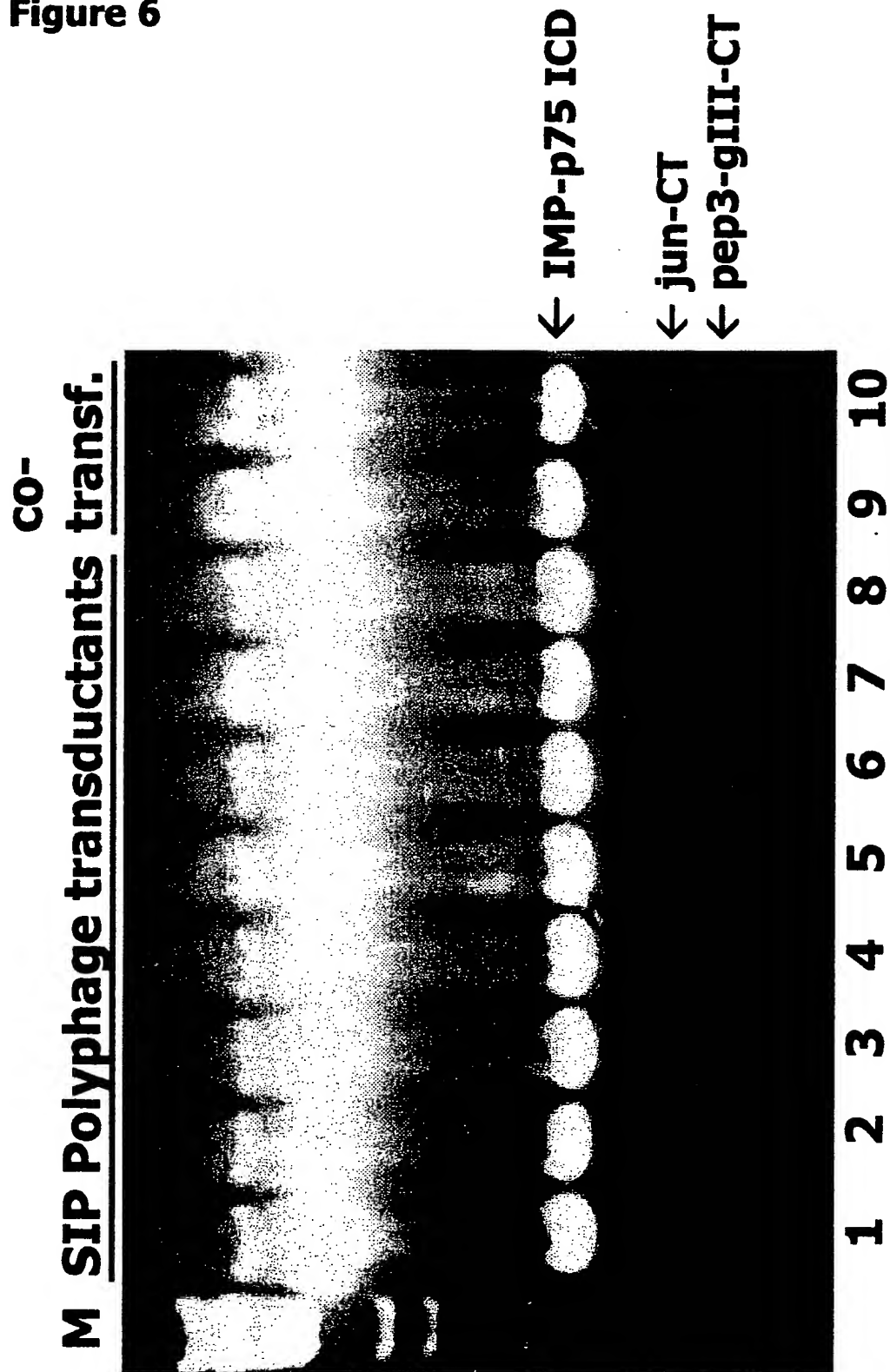
**Figure 5**



SUBSTITUTE SHEET (RULE 26)

35/39

**Figure 6**



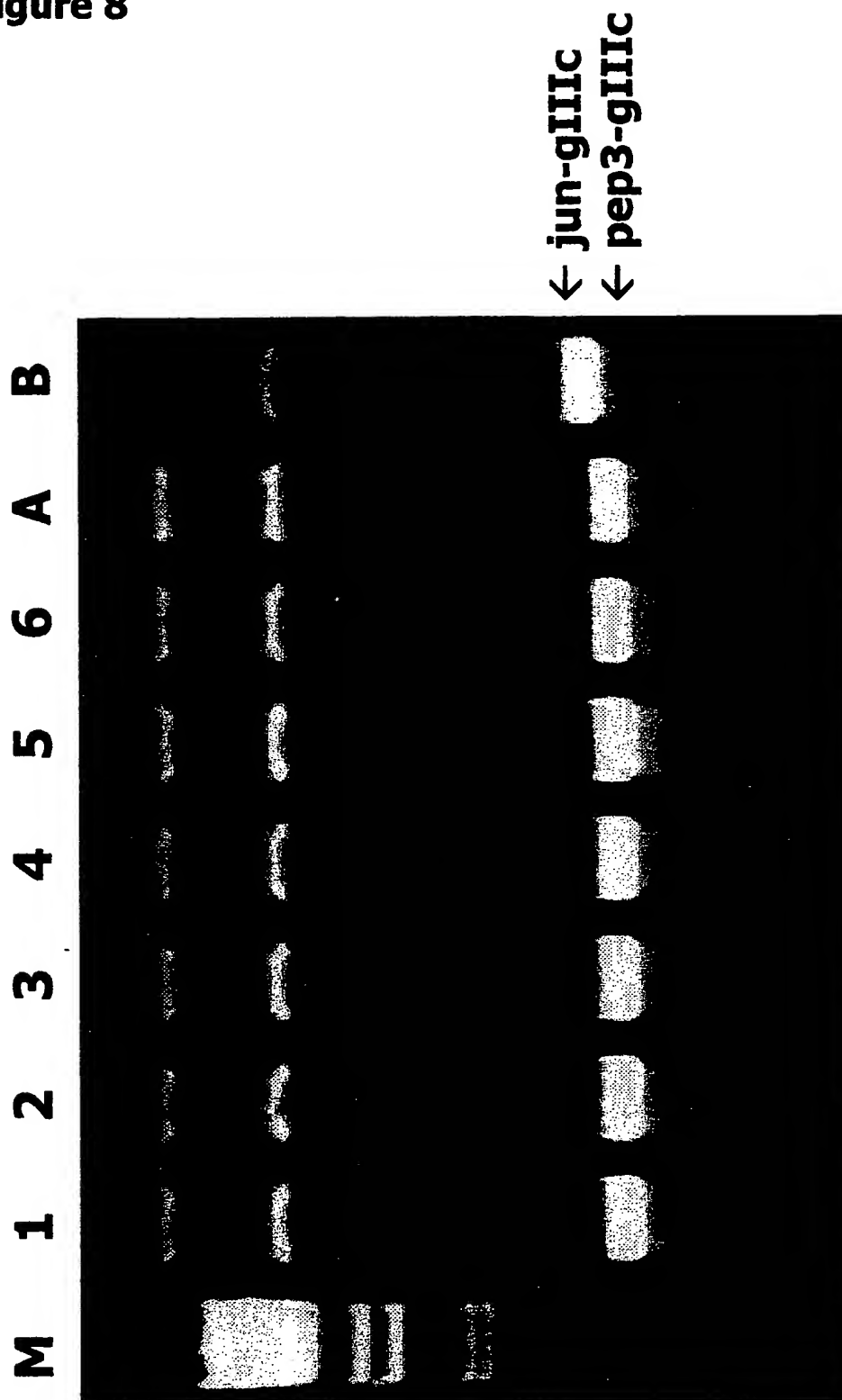
36/39

**Figure 7**

| <b>pep3/p75ICD</b> |                     | <b>dilution factor</b>   |  | <b>transductants</b>                |  |
|--------------------|---------------------|--------------------------|--|-------------------------------------|--|
|                    |                     | <b>jun/p75ICD</b>        |  | <b>(t.u./ml)*</b>                   |  |
| <b>1</b>           | <b>pos. control</b> | <b>-</b>                 |  | <b><math>6 \times 10^5</math></b>   |  |
| <b>-</b>           | <b>neg. control</b> | <b>1</b>                 |  | <b>0</b>                            |  |
| <b>1</b>           |                     | <b><math>10^2</math></b> |  | <b><math>1.2 \times 10^4</math></b> |  |
| <b>1</b>           |                     | <b><math>10^3</math></b> |  | <b><math>8.6 \times 10^2</math></b> |  |
| <b>1</b>           |                     | <b><math>10^4</math></b> |  | <b><math>1.2 \times 10^2</math></b> |  |
| <b>1</b>           |                     | <b><math>10^5</math></b> |  | <b><math>12^{\#}</math></b>         |  |
| <b>1</b>           |                     | <b><math>10^6</math></b> |  | <b><math>1.2^{\#}</math></b>        |  |
| <b>1</b>           |                     | <b><math>10^7</math></b> |  | <b><math>0.12^{\#}</math></b>       |  |

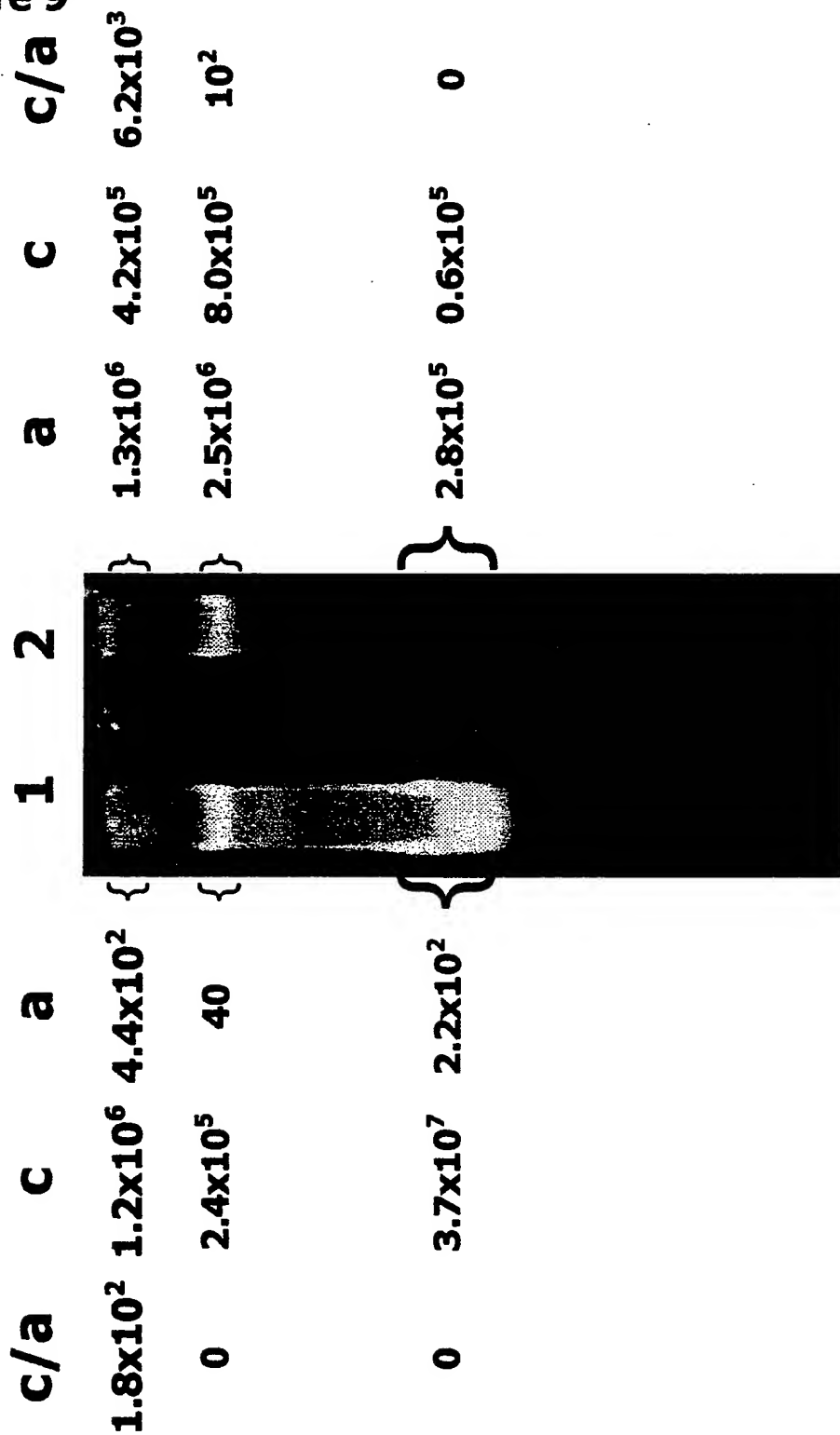
37/39

**Figure 8**

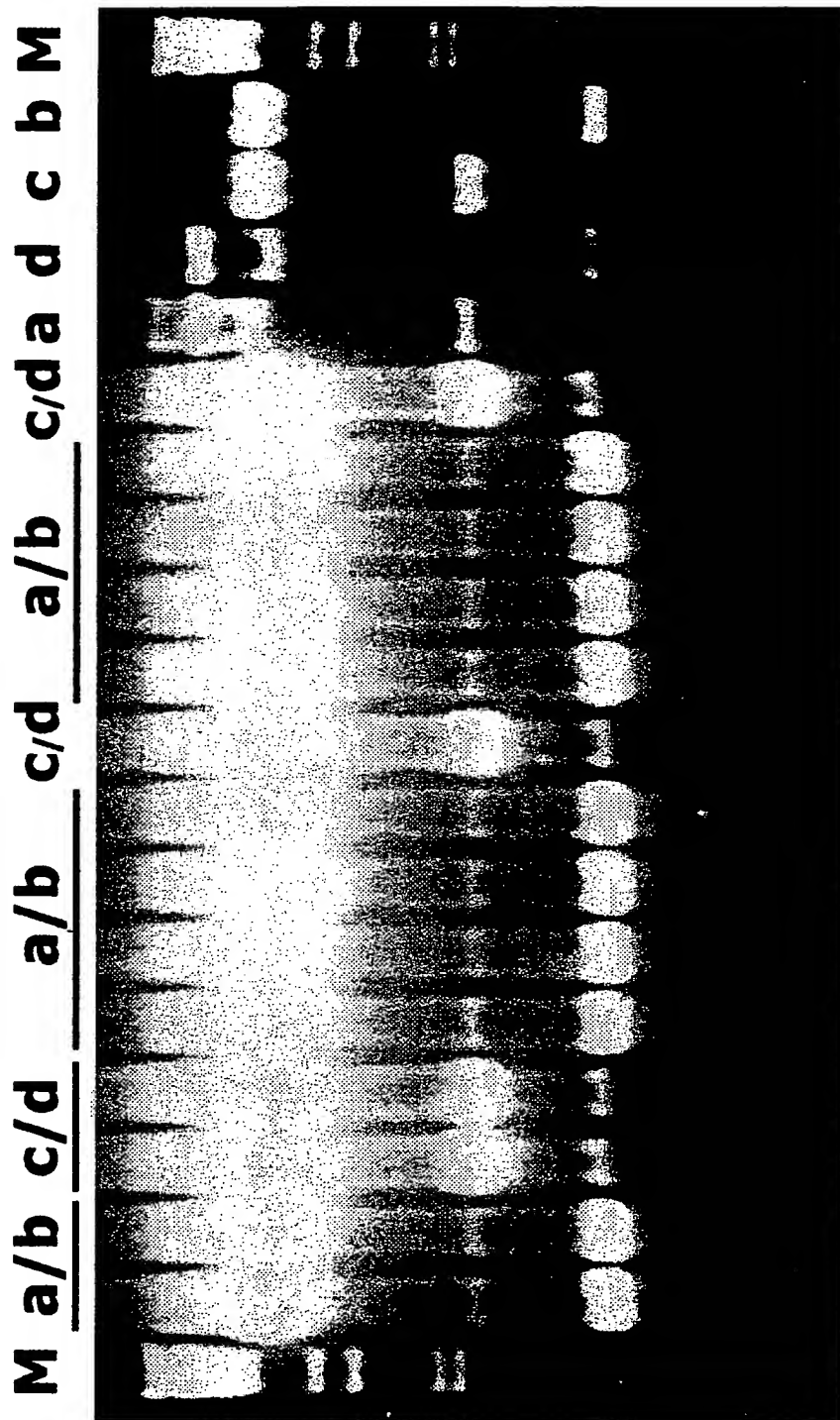


38/39

Figure 9



39/39

**Figure 10**



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**